University Of Alberta

Nutritional Status, with Particular Reference to Vitamin A, of First Nation Adults With and Without Non-Insulin Dependent Diabetes Mellitus.

By

Carlota Grace Basualdo

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in partial fulfillment of the requirements for degree of
Master of Science

In

Nutrition and Metabolism

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C Basualdo

Carlota Basualdo
263 Westridge Road
Edmonton, Alberta Canada
T5T 1C2

Date: July 30, 1996

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *the nutritional status, with particular reference to vitamin A, of First Nation adults with and without non-insulin dependent diabetes mellitus*, submitted by *Carlota Grace Basualdo* in partial fulfillment of the degree of *Master of Science* in *nutrition and metabolism.*

Dr. T.K. Basu, Supervisor

Dr. C.J. Field

Dr. L.J. McCargar

Dr. R.V. Rajotte

Dr. E. Wein

Date: July 05/96

ABSTRACT

The vitamin A status of Cree adults (> 40 years; M=41, F=71) residing in central Alberta with non-insulin dependent diabetes mellitus (NIDDM) (n=62) was compared to non-diabetic controls (n=50). The specific objectives were to: 1) assess vitamin A and retinol binding protein (RBP) status, along with factors that may affect their status (i.e., nutritional status, plasma insulin levels, and metabolic control); and 2) assess the nutritional status of First Nation adults from 24-hr food recalls, a vitamin A food frequency, and anthropometric measures. Subjects with NIDDM had central obesity, hyperglycemia, and hypertriglyceridemia. Vitamin A status was normal in subjects with NIDDM, although plasma RBP was elevated. Many individuals were at risk of inadequate intakes of vitamin A, D, calcium, folate and zinc; however, those with NIDDM reported healthier dietary patterns. Conclusions were: 1) vitamin A status is not a concern in NIDDM with insulin sufficiency, and 2) nutrition and diabetes education should be extended to the whole community.

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LIST OF ABBREVIATIONS

BMI	body mass index
cRBP	cellular retinol binding protein
DM	diabetes mellitus
IDDM	insulin dependent diabetes mellitus
MJ	megajoule
MUFA	mono-unsaturated fatty acid
NIDDM	non-insulin dependent diabetes mellitus
PUFA	poly-unsaturated fatty acid
RBP	retinol binding protein
RNI	recommended nutrient intake
SFA	saturated fatty acid
TTR	transthyretin
WHR	waist hip ratio

1. INTRODUCTION

Overview of Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disease characterized by inadequate production or utilization of insulin. Insulin is a hormone produced by the β-cells of the pancreas and promotes uptake of glucose by its sensitive tissues, which include adipose tissue and skeletal muscle. Clinical manifestations of DM include hyperglycemia, glycosuria, and altered carbohydrate, fat and protein metabolism. Chronic complications resulting from macro- and micro-pathology include cardiovascular disease, nephropathy, neuropathy, and retinopathy followed by blindness.

There are two major types of DM: insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). Insulin dependent diabetes mellitus is an autoimmune disease that involves β-cell destruction and subsequent insulin deficiency. It occurs more frequently during childhood years. In contrast, NIDDM is more prevalent in adults and obese individuals. A striking difference is that individuals with NIDDM may actually have high blood insulin levels, a consequence of reduced insulin sensitivity or insulin resistance (Olefesky and Nolan, 1995). To compensate for insulin resistance, more insulin is released, resulting in hyperinsulinemia. Prior to onset of NIDDM, there may be adequate insulin released despite the presence of insulin resistance. Non-insulin dependent diabetes mellitus develops when the β-cells in the pancreas fail to release enough insulin to compensate for the insulin resistance. Hyperglycemia occurs due to a combination of increased hepatic glucose output and insufficient insulin to normalize blood sugar levels. There is a multitude of factors, both environmental and genetic, that may lead to insulin resistance and β-cell failure (Olefesky and Nolan, 1995). Non-insulin dependent diabetes mellitus is a heterogeneous disease and its pathogenesis differs between individuals and among cultures.

Treatment for IDDM and NIDDM also differs, although diet modifications and

exercise are often essential in the treatment of both. Individuals with IDDM require daily insulin injections. In contrast, treatment for NIDDM may involve oral hypoglycemic agents and/ or insulin injections. Weight loss may also improve metabolic control of NIDDM (Tinker et al., 1994).

Vitamin A and Diabetes Mellitus

Vitamin A is necessary for the light-sensitive pigments in the eye that enable night vision. In addition, it helps to maintain the integrity of epithelial tissues and thereby prevent infections. Vitamin A may be especially important for people with DM, since this disease is associated with both retinopathy and an increased severity of infections (Rayfield et al., 1982; Patel et al., 1991). A commonality of vitamin A deficiency and diabetic retinopathy is that both can cause blindness. A hypothesis that vitamin A metabolism may be altered in DM originated from reports of hypercarotenemia among patients with DM (Boeck and Yater, 1929; Rabinowitch, 1930; Ralli et al., 1935; Heyman; 1936). More recent research has suggested that DM, especially when poorly controlled, may lead to altered metabolic availability of vitamin A (Wako et al., 1986; Basu et al., 1989; Krempf et al., 1991; Martinoli et al., 1993).

Blood vitamin A levels are normally maintained at a fairly constant level despite normal fluctuations in daily intake. Dietary sources of vitamin A include preformed retinyl esters from animal sources and provitamin A, carotenoid from plant sources. In the intestine, retinyl esters are hydrolyzed to retinol by pancreatic lipase before absorption (Blomhoff et al., 1991). Fat stimulates pancreatic lipase secretion; thus the amount of retinol absorbed is influenced by the quantity of fat in the diet. In the enterocytes, retinol is re-esterified and incorporated into chylomicrons together with triglycerides. The chylomicrons carry the retinyl esters mainly to the liver, where they are stored predominantly in the stellate cells. Retinyl esters are hydrolyzed to retinol,

which then binds to retinol binding protein (RBP), and is released into circulation to be transported to target tissues. Before reaching the target tissues, however, retinol bound to RBP forms a complex with transthyretin (TTR) in the circulation, which increases the stability of RBP and prevents its catabolism. At the target tissues, the TTR is released and RBP facilitates the uptake of retinol by the RBP receptors. Retinol binds with cellular retinol binding protein (cRBP) which facilitates transport of retinol within and between cells. Retinol may be esterified and stored or may be converted to more active metabolites such as retinoic acid (Norum and Blomhoff, 1992).

Early reports of hypercarotenemia in DM were proposed to be a consequence of impaired conversion of β-carotene to vitamin A (Ralli et al., 1935, 1936). This hypothesis was supported by the observation that a loading dose of carotene, administered to those with DM, caused the blood carotene to rise to a higher level and remain there longer than in controls (Ralli et al. , 1936; Heymann, 1936). In contrast, other investigators reported a normal rise in blood carotene after a loading dose, normal conversion rates of carotene to vitamin A, and normal blood vitamin A concentrations in those with DM compared to controls (Murrill et al., 1941). A later study found wide intersubject variability in plasma vitamin A and carotene concentrations and a trend towards a low ratio of vitamin A to carotene (Kimble et al., 1946). High blood carotene with deficient vitamin A or evidence of carotenemia was uncommon (Kimble et al., 1946). Conflicting results may be due to differences in the study participants regarding type of diabetes, metabolic control of diabetes, diet, and presence of diabetic complications or other diseases. As the management of DM progressed, hypercarotenemia among patients with DM became less frequent (Mosenthal et al., 1944).

Recent literature has reexamined vitamin A metabolism in DM (Table 1.1). Patients with IDDM have lower plasma concentrations of retinol than non-diabetic controls (Wako et al., 1986; Basu et al., 1989; Krempf et al., 1991; Martinoli et al., 1993),

although mean plasma concentrations were in the normal range. Serum vitamin A levels greater than 1.05 μmol/L are indicative of adequate vitamin A status (Gibson, 1990). When the subjects were stratified by age, only the younger subjects with IDDM had low retinol levels compared to controls (Martinoli et al., 1993). Although retinol levels were significantly lower in those with IDDM compared to controls, plasma retinyl esters in the lipoprotein fractions of fasting blood samples were higher (Wako et al., 1986). Lipoprotein fractions may act as a nonspecific transport of vitamin A to target tissues. Unlike in patients with IDDM, vitamin A status does not appear to be diminished in patients with NIDDM, as indicated by some isolated reports (Table 1.1). In fact, retinol levels were found to be higher in patients with NIDDM than controls (Havivi et al., 1991; Krempf et al., 1991). Another study of patients with NIDDM compared patients with and without neuropathy to healthy controls and reported normal plasma vitamin A and β-carotene concentrations (Straub, 1993).

Streptozotocin-induced DM in adult male rats also resulted in decreased plasma and increased hepatic retinol concentrations compared to control rats (Basu et al., 1990; Leichter et al., 1991; Tuitoek et al., 1996a). A decrease in the concentration of 11-cis-retinal, a constituent of rhodopsin, in the retina was also reported in parallel with a decrease in the circulating levels of retinol in diabetic rats (Tuitoek et al., 1996a).

Table 1.1. Reported plasma retinol concentrations in subjects with and without diabetes mellitus.

Study	Plasma Retinol Concentration (μmol/L)		*p*
IDDM	People with diabetes	Controls	
Wako et al., 1986	1.68 ± 0.83[1] (49)[2]	2.32 ± 0.36 (12)	< 0.01
Basu et al., 1989	1.63 ± 0.33 (25)	1.86 ± 0.35 (25)	< 0.05
Krempf et al., 1991	1.20 ± 0.30 (35)	1.77 ± 0.30 (30)	< 0.0001
Martinoli et al., 1993	1.45 ± 0.36 (44)	1.82 ± 0.45 (18)	< 0.01
NIDDM			
Havivi et al., 1991	1.96± 0.51 (100)	1.73 ± 0.30 (112)	<0.001
Krempf et al., 1991	2.16 ± 0.96 (35)	1.77 ± 0.30 (30)	< 0.001

1. *Mean ± S.D.*
2. *Figures in parentheses indicate number of subjects in study group*

Factors Affecting Circulating Retinol

Factors that may affect blood vitamin A levels in people with DM include: 1) dietary factors; and 2) levels of retinol carrier proteins. Dietary intake of vitamin A and fat may influence vitamin A status by influencing levels of pancreatic lipase, necessary for the hydrolysis of retinyl esters, and palmitate, the major lipid for synthesis of retinyl esters (Blomhoff et al., 1991). In addition, hyperlipidemia may cause retinol levels to

increase independently of RBP (Smith et al., 1992). In individuals with chronic renal disease, for example diabetic nephropathy, levels of both vitamin A and RBP and their molar ratio may be elevated (Smith and Goodman, 1971). Retinol binding protein, however, may have the greatest influence on circulating vitamin A levels. Retinol binding protein has a short biological half life (approximately 12 hours), and may be affected by numerous parameters, including vitamin A and zinc status, protein status, age, gender, and trauma (Rask et al., 1980).

Circulating levels of RBP may be altered in DM. Retinol binding protein levels have been shown to be reduced in IDDM (Basu et al., 1989; Kemp and Frindik, 1991). Another study demonstrated diminished circulating levels of RBP, which did not reach significance, and significantly lower concentrations of TTR in IDDM compared to controls (Mohan et al., 1991). Streptozotocin-induced diabetic rats have also shown reduced RBP and TTR levels in plasma, as well as in the kidney (Tuitoek et al., 1996b). These results suggest that transfer of retinol to the target tissues may be of concern in DM. The mechanism causing altered RBP levels in DM is not known. Several factors may contribute to altered RBP levels in DM including hyperzincuria, high renal loss of RBP, levels of plasma insulin, and metabolic control of diabetes. The effect of these factors on RBP status will be described.

Hyperzincuria

Zinc deficiency is known to reduce the synthesis and release of RBP into circulation, resulting in increased hepatic vitamin A level and an impaired metabolic transport (Smith et al., 1973; Smith et al., 1974; Brown et al., 1976; Michaelsson et al., 1976; Shingwekar et al., 1979). More recent work also found that cellular RBP was reduced in zinc deficient rats, suggesting impaired intracellular transport of vitamin A (Mobarhan et al., 1992). Hepatic enzymes regulating vitamin A status may be altered by

zinc deficiency (Sundaresan et al., 1977; Boron et al., 1988). In zinc deficiency, levels of retinal oxidase, the enzyme that regulates the irreversible oxidation of retinal to retinoic acid, are significantly increased (Sundaresan et al., 1977; Boron et al., 1988) and hepatic retinol dehydrogenase is decreased compared to controls (Boron et al., 1988).

Diabetes mellitus may be associated with a greater risk of zinc deficiency. Hyperzincuria has been reported in individuals with NIDDM (Kinlaw et al., 1983; Schlienger et al., 1988; Melinkeri et al., 1990; Walter et al., 1991; Car et al., 1992; Honnorat et al., 1992) and IDDM (Hägglöf et al., 1983; Heise et al., 1988; Schlienger et al., 1988; Mocchegiani et al., 1989; Walter et al., 1991; Brun et al., 1992; Car et al., 1992; Honnorat et al., 1992). Total body zinc clearance was higher in children with IDDM than controls and was associated with shorter stature (Nakamura et at., 1991). Increased urinary loss of zinc has been associated with hypozincemia by some researchers (Kinlaw et al., 1983; Schlienger et al., 1988; Mocchegiani et al., 1989; Melinkeri et al., 1990; Walter et al., 1991; Car et al., 1992), but not by others (Heise et al, 1988; Brun et al., 1992; Honnorat et al., 1992).

The mechanism of hyperzincuria in DM appears to be multifactoral. Zinc appears to have a high affinity for chelation with sugar-amine complexes, and an increase in glycosylated amino acids and peptides may contribute to elevated urinary zinc excretion (Heise et al., 1988). Most researchers, however, have reported that zinc deficiency did not correlate with glycemic control as indicated by levels of glycosylated hemoglobin (HbA1C) or serum glucose (Hägglöf et al., 1983; Kinlaw et al., 1983; Heise et al., 1988; Mocchegiani et al., 1989; Walter et al., 1991; Brun et al., 1992; Honnorat et al., 1992).

Zincuria may be related to blood insulin levels. Insulin treatment causing hyperinsulinemia, in patients with NIDDM, was associated with reduced hyperzincuria compared to both NIDDM patients treated without insulin and IDDM patients

(Honnorat et al, 1992). Furthermore, insulin therapy in children with IDDM was associated with a normalization of zinc concentration in both serum and blood clots within one month (Hägglöf et al., 1983). This is in agreement with earlier studies that found that insulin inhibited urinary zinc excretion (Tarui, 1963; Lau and Failla, 1984).

Incipient diabetic nephropathy has been suggested to be a potential cause of zinc loss. Patients with excessive microalbuminuria had significantly higher zinc excretion, although zincuria and microalbuminuria were not correlated (Hägglöf et al., 1983; Brun et al., 1992; Honnorat et al., 1992). Zinc excretion correlated with total protein loss; hence zinc excretion may be related to increased tissue catabolism during fasting (e.g., overnight) (Heise et al., 1988). Diabetic renal disease does not appear to be the primary cause of hyperzincuria, but may aggravate it.

Excretion of Retinol Binding Protein

Proximal tubular dysfunction is a complication of DM, and is indicated by excretion of low molecular weight proteins. Retinol binding protein has a low molecular weight and circulates in plasma where the majority is bound to TTR and a small portion is unbound. Normally, the unbound fraction is filtered by the glomeruli and catabolized after reabsorption in the proximal tubules; at this point a small amount may leak into the urine (Rask et al., 1980; Bernard et al., 1982). In proximal tubular dysfunction, large amounts of RBP may be excreted.

Excretion of RBP is increased in both IDDM (Rowe et al., 1987; Watts et al., 1989; Bernard et al., 1990; Pontuch et al., 1992; Catalano et al., 1993) and NIDDM (Bernard et al., 1990; Holm et al., 1993; Koh et al., 1993; Holm et al., 1994). An increased urinary excretion of RBP has been found in normoalbuminuric diabetic patients. This indicates that proximal tubular dysfunction occurs independently of glomerular dysfunction (indicated by microalbuminuria) and overt diabetic nephropathy in patients

with IDDM (Rowe et al., 1987; Watts et al., 1989; Bernard et al., 1990; Pontuch et al., 1992; Catalano, 1993) and NIDDM (Holm et al., 1993; Holm et al., 1994). Retinol binding protein excretion, nevertheless, has been weakly correlated with albuminuria in some studies of IDDM (Rowe et al., 1987; Bernard et al., 1990), but not in others (Pontuch et al., 1992). Albuminuria was also found to correlate with RBP excretion in NIDDM (Koh et al., 1993).

Excretion of RBP correlated with long-term metabolic control in both IDDM (Pontuch et al., 1992) and NIDDM (Holm et al., 1993; Koh et al., 1993). However, other studies involving patients with IDDM found no correlation between metabolic control or fasting insulin levels and excretion of RBP (Rowe et al., 1987; Watts et al., 1989; Catalano et al., 1993).

It is not known if RBP excretion in subjects with DM results in diminished levels of serum RBP. One study examined serum levels of RBP to exclude 'tubular overflow proteinuria' as a cause of increased RBP excretion in NIDDM; the data were not reported, although the researchers did report that no patient had an above normal serum RBP concentration (Holm et al., 1993).

Plasma Insulin

Both subcutaneous insulin injections (Leichter et al., 1991) and implantible insulin administration (Tuitoek et al., 1996c) normalized plasma concentrations of vitamin A and RBP in experimental animals. Insulin is known to be one of the most important anabolic regulators of protein metabolism. A lack of insulin, as occurs in uncontrolled IDDM, results in muscle wasting that is reversed by insulin treatment. A study examining whole body protein turnover in patients with IDDM found that poor metabolic control resulted in both increased protein breakdown and synthesis, with a net protein loss (Nair et al., 1983). Due to limitations of the methodologies used, there is still some uncertainty about

the effect of DM on protein synthesis in the liver (Nair et al., 1983). Further studies are required to determine the effect of insulin on hepatic RBP, although indirect evidence indicates that insulin deficiency causes less RBP to be released from the liver (Leichter et al., 1991; Tuitoek et al., 1996c).

Less is known about protein metabolism in NIDDM. Some investigators have reported that, although insulin mediated glucose disposal is significantly impaired in NIDDM, the effect of insulin on protein degradation and protein synthesis is normal in DM with normoglycemia (Staten et al., 1986; Welle and Nair, 1990; Biolo et al., 1992; Luzi et al., 1993; Pijl et al., 1994) The regulation of protein turnover may be normally sensitive to insulin in NIDDM, even though glucose disposal is resistant to insulin. Results of a study of adults with NIDDM and moderate hyperglycemia showed abnormal protein metabolism: obese individuals with NIDDM had greater whole-body nitrogen flux, protein synthesis, protein breakdown, and resting energy expenditure compared to obese controls (Gougeon et al., 1994). Net protein loss was significantly lower for hyperglycemic subjects than euglycemic subjects consuming the same diet (Gougeon et al., 1994). Poorly controlled patients with NIDDM also have higher urea synthesis, which was partially normalized with improvement of metabolic control (Almdal et al., 1994). Individuals with NIDDM may have low, normal or high blood insulin levels, and therefore the effect of NIDDM on RBP levels may be variable depending on metabolic control and blood insulin levels.

Metabolic Control of Diabetes Mellitus

Improvement of the metabolic control of IDDM in children improved serum concentrations of transport proteins, including RBP and TTR, although no correlations between changes in the glycated markers and serum protein levels were found (Kemp and Frindik, 1991). Metabolic control of DM could influence RBP levels by several

mechanisms. As discussed previously, hyperzincuria, RBP excretion, and insulin levels are all influenced by metabolic control and may contribute to altered levels of RBP. Poor metabolic control of DM is also associated with increased severity and incidence of infections (Rayfield et al., 1982; Patel et al., 1991) and vascular tissue damage due to hyperglycemia, which in turn may negatively affect RBP concentrations. Infection or tissue damage may result in lower levels of RBP, as a consequence of the acute phase response. The acute phase response is defined as "an early and unspecific but highly complex reaction of the animal organism to a variety of injuries such as bacteria or parasitic infection, mechanical or thermal trauma, malignant growth or ischaemic necrosis" (Koj and Gordon, 1985). Retinol binding protein levels have been reported to be depressed in the acute stage of measles (Reddy et al., 1986). It is not known if an acute phase response to infection or tissue trauma in people with DM results in altered levels of RBP.

Summary of Potential Mechanisms for Altered Retinol Binding Protein Levels in Diabetes Mellitus

Evidence to date suggests that insulin is the major factor responsible for altered availability of vitamin A in DM. Reduced circulating retinol in IDDM occurs predominately as a result of insulin deficiency which in turn reduces plasma concentrations of vitamin A carrier proteins. Insulin administration, in experimental models of IDDM, normalizes both plasma and liver retinol concentrations (Leichter et al., 1991; Tuitoek et al., 1996c). Depressed plasma retinol concentrations do not appear to be due to malabsorption (Tuitoek et al., 1994). Furthermore, zinc supplementation failed to improve plasma concentrations of vitamin A (Tuitoek et al., 1996c). Excretion of RBP also does not appear to be the major factor causing reduced plasma concentrations of RBP, although it may be a contributor. The acute phase response may also contribute to

reduced RBP levels, but does not appear to be the major mechanism involved. Further research is required to clarify the mechanism causing depressed concentrations of vitamin A and RBP, and the contribution of the various factors discussed.

Subclinical Deficiency of Vitamin A

Although circulating concentrations of vitamin A in patients with DM have been found to be significantly lower than in control subjects, vitamin A levels remained within the normal range (Basu et al., 1989; Wako et al., 1986; Martinoli et al., 1993). Vitamin A levels were not depressed severely enough to produce overt signs of vitamin A deficiency. Nevertheless, marginal vitamin A levels may affect the health of these patients.

Subclinical vitamin A deficiency may impair the immune response and delay wound healing. Subclinical deficiency in children in developing countries is associated with higher rates of morbidity and mortality from infections (Sommer et al., 1983; Bloem et al., 1990). In developed countries, the occurrence of a marginal state of vitamin A deficiency is being recognized (Newman et al., 1994). Vitamin A is known to promote wound healing (Brandaleone and Papper, 1941), and administration of vitamin A has been beneficial postoperatively (Cohen et al., 1979).

Vitamin A may improve the immune response by regulating cellular differentiation in epithelial tissue. Mild vitamin A deficiency may lead to keratinization of the epithelial lining of the respiratory, genitourinary, and gastrointestinal tracts (Rumore, 1993). These barriers are the body's first line of defense against infection. Many aspects of the non-specific and specific responses to infection may be affected by vitamin A deficiency (West et al., 1991; Rumore, 1993).

Individuals with DM often have a higher prevalence and severity of infection; thus adequate vitamin A status may prove beneficial. Supplementation with vitamin A enhanced wound healing in streptozotocin-induced diabetic rats (Seifter et al., 1981).

Studies are required to determine if observations of significantly lower circulating levels of vitamin A in subjects with DM compared to controls are of clinical significance.

It is not known if subclinical vitamin A deficiency aggravates development of retinopathy leading to blindness. Poor metabolic control of DM accelerates the progression of retinopathy (Porta, 1993; Stolk et al., 1995) and also results in reduced serum vitamin A levels (Wako et al., 1986; Basu et al., 1989; Martinoli et al., 1993). Moreover, patients with diabetes and retinopathy had significantly higher excretion of RBP but not albumin compared to controls (Holm et al., 1994). Further research is required to determine if there is a link between subclinical vitamin A deficiency and retinopathy in DM.

Diabetes Mellitus in Aboriginal People

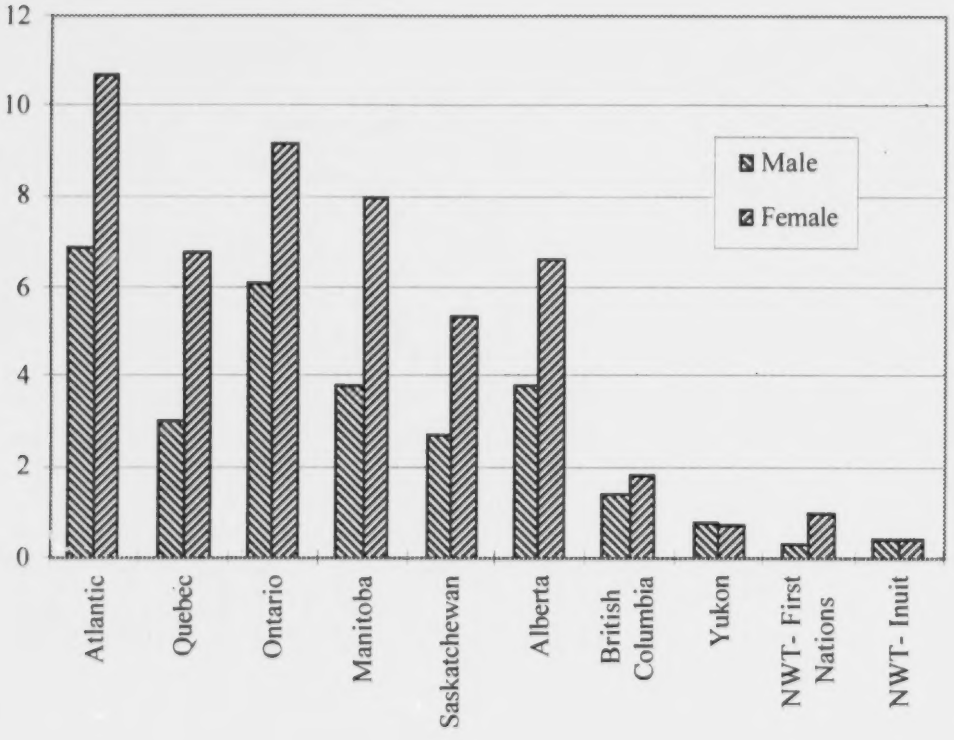
An almost unknown condition among aboriginal people prior to the 1940s, NIDDM has recently risen in epidemic proportions in many aboriginal population groups. There are several recent reviews of the epidemiology and etiology of NIDDM in indigenous groups in Canada (Young et al., 1992; Young, 1993; Szathmáry, 1994) and elsewhere including the United States (Gohdes et al., 1993), Australia (O'Dea, 1991) and the South Pacific (Zimmet et al., 1990).

The prevalence of diagnosed DM for 76 % of the registered Indian and Inuit population of Canada was determined (Figure 1.1); the age-sex adjusted rate varied among the various aboriginal groups within Canada from a low of 0.8 % in the Northwest Territories to a high of 8.7 % in Atlantic Canada (Young et al., 1990). Latitude accounted for 30.8 % of the variation in DM prevalence. Language phylum-culture combinations, which indicate genetic background, were the next most important predictors of variation in DM prevalence. The distribution of DM among Canadian aboriginal peoples provides evidence that both genetic and environmental factors are important determinants of risk of

DM.

Models explaining the high prevalence of NIDDM in indigenous peoples have been proposed, beginning with the "thrifty gene" theory (Neel, 1962). This theory suggests that, during earlier times characterized by a traditional lifestyle of hunting and gathering, people with genes that enabled them to convert excess glucose to fat for storage efficiently during times of feasting (i.e., so called "thrifty genes") would have a selective advantage. The "New World Syndrome" links lipid and carbohydrate metabolism, and suggests that people with genes that promote more efficient storage of fat during feasting and release of fat during fasting would be more likely to survive and reproduce (Weiss et al., 1984). Both the thrifty gene and the New World models imply that the traditional diet included a high proportion of carbohydrate during times of feasting. This was not the case within Canada, especially northern Canada where the diet consisted predominantly of meat and fat. A model suggesting an adaptation to a low carbohydrate diet and a cold environment was proposed (Szathmáry, 1990). It suggested that genes which promote gluconeogenesis and free-fatty acid release and utilization would enhance survival in this environment. A further theory linked the low carbohydrate adaptation and the New World models: the Northern Hunting Adaptation proposed a genetic advantage involving several enzymatically mediated pathways involving glucose and lipid metabolism (Ritenbaugh and Goodby, 1989).

Figure 1.1. Regional variation in age-standardized prevalence of diabetes among Canadian aboriginals (Young, 1990).



For the hunter-gatherers, whose diet consisted largely of protein, hyperinsulinemia and insulin resistance would promote efficient fat accumulation during times of feasting. Metabolic changes, including the active conversion of dietary protein to glucose and then to fat, insensitive to suppression by insulin, may have evolved (O'Dea, 1991). Traditionally, indigenous people were lean and physically active, two conditions which promoted insulin sensitivity in the presence of hyperinsulinemia. With a more modern lifestyle, these adaptations have led to a constant rise in blood insulin and glucose

levels, resulting in obesity, an important risk factor for NIDDM. The modern diet is high in energy, fat and carbohydrate, and produces a high glycemic and insulin response. Hepatic glucose output is not suppressed by insulin in the normal way and this exacerbates the problem. Glycemia is maintained for a longer time, which promotes triglyceride and VLDL synthesis in the liver and fat accumulation in adipose tissue (O'Dea, 1991). Both glycemia and obesity promote further insulin resistance, and hyperinsulinemia becomes more extreme. Non-insulin dependent diabetes mellitus results in those individuals who are no longer able to compensate for the insulin resistance. Insulin resistance has been demonstrated to be part of the etiology of NIDDM in indigenous groups world-wide including the Pima Indians of Arizona (Bogardus, 1993), the Australian Aborigines (O'Dea, 1991), and indigenous people from the South Pacific (Zimmet et al., 1990).

Diabetes mellitus has become a major health problem for indigenous peoples and involves a high prevalence and severity of complications. Both Canadian and American national surveys have demonstrated an increased mortality from DM among indigenous peoples (Young, 1993). In Canada, on Indian reserves in seven provinces from 1977-1982, the risk of death from DM was 2.2 times higher among men and 4.1 times higher among women than among Canadian men and women in general (Mao et al., 1986). First Nations people with NIDDM suffer from a high rate of complications including end-stage renal disease, lower-extremity amputations, retinopathy and blindness, and cardiovascular disease, which is higher than the general population with NIDDM (Young, 1993).

Purpose and Objectives of Proposed Study

Evidence has been presented that convincingly demonstrates a link between DM and alterations in the metabolic availability of vitamin A. Diabetes mellitus is associated with several factors which may contribute to lower plasma concentrations of vitamin A

and its carriers, of which insulin deficiency appears to be the major mechanism. The majority of the research has examined IDDM. It is not clear if circulating levels of vitamin A and its carrier proteins are altered in NIDDM. Individuals with NIDDM may have low to high insulin levels depending on the duration of DM and its metabolic control. Early stages of NIDDM, with mild glycemia and retained ability to compensate somewhat for the insulin resistance, are often associated with hyperinsulinemia. In later stages of NIDDM with severe hyperglycemia, relative insulin deficiency may be evident. At the different stages of NIDDM, it is possible that the influence of the diabetic state on vitamin A metabolism may vary.

The prevalence of NIDDM has increased dramatically in the past 20 years in the Canadian indigenous population, and now for many First Nation groups, greatly exceeds that of the general population (Young, 1993). Within Canada, regional differences are apparent: diabetes is more prevalent among aboriginal groups in the southern and eastern areas of Canada, whose environment and lifestyle are more urban in nature than groups in western and northern Canada (Young et al, 1990). Circumpolar indigenous populations generally have the lowest prevalence (Young et al, 1992).

The high prevalence of NIDDM in First Nation communities provides an opportunity to examine its effect on vitamin A metabolism. Moreover, a nutritional study in a First Nation community would provide valuable health information that could be used for health planning and education.

Evidence from indigenous communities world-wide suggests that Canadian First Nation people are susceptible to insulin resistance, and this is contributing to the increased prevalence of both obesity and NIDDM. A high prevalence of complications from DM has been documented in First Nation communities in Canada. This may be partly due to difficulty in maintaining good metabolic control, a result of a combination of factors including inadequate education programs and resources, and difficulty in adopting

the necessary dietary and lifestyle changes. Furthermore, First Nation people often develop NIDDM at an earlier age than non-native people, and complications occur with increasing duration of the disease.

In view of the poor metabolic control and early onset of NIDDM in Canadian aboriginal communities, it was hypothesized that a select group of Plains Cree adults with NIDDM in central Alberta would have impaired transport of vitamin A. The specific objectives of this study were to:

1) determine if Plains Cree adults with NIDDM have reduced vitamin A and RBP status compared to non-diabetic controls;

2) assess the influence of factors that may affect RBP status including zinc status, protein status, plasma insulin levels, and metabolic control of diabetes; and

3) determine the nutritional status of persons with diabetes and non-diabetic controls from three non-consecutive 24-hr food recalls, a vitamin A food frequency questionnaire and anthropometric measures.

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2. STUDY POPULATION

Geographic Location and Demographics

Subjects were recruited from four bands of Plains Cree residing in central Alberta, namely the Ermineskin, Louis Bull, Montana, and Samson First Nations. The bands reside on six large reserves encompassing 74,612 acres located 13 to 76 km south and west of Wetaskiwin, Alberta (Indian and Northern Affairs Canada, 1995). The bands are engaged in farm and ranch operations, oil and gas extraction, construction, retailing, education, health and social services, as well as band administration. Although they occupy a large rural ranching area, members have rapid and easy access to major urban centres. Health care services, including home care and health education programs, are provided mainly through the Hobbema Indian Health Services (Health Center), which is jointly band and federally operated. Hospital services, dietetic counselling, and specialist medical referrals are provided by services in the town of Ponoka and the cities of Wetaskiwin and Edmonton.

The total population of the area is 9019 persons (4439 male and 4580 female), with 7431 persons living on the reserve (Indian and Northern Affairs Canada, 1995). The prevalence of diabetes in this population is not known. However, in a national survey of the aboriginal population of Canada (1987), the age adjusted prevalence rate of diabetes for Alberta's First Nations people was 5.1 % (3.8% for males, 6.6% for females), placing them about mid way on the range of prevalence of diabetes among Canadian aboriginal people regionally (Figure 1.1) (Young et al., 1990). Among the 38 350 Alberta people who identified themselves as North American Indian in the 1991 census, 2 005 persons or 5.2 % reported the presence of diabetes (Statistics Canada, 1995).

Study Subjects

Men and women of Indian legal status (i.e., held band membership), age 40 years and older were recruited, with the help of local aboriginal research assistants who spoke English and Cree. Information about the study was posted in doctor offices, the Health Center, the band offices and schools. Potential subjects were contacted by telephone or, for those who did not have a telephones, home visits were made. Persons with non-insulin dependent diabetes mellitus (NIDDM) were recruited from a list of people known to have diabetes provided by Health Centre. Physicians in Wetaskiwin and Ponoka also recruited a few participants. Persons without NIDDM (controls) were recruited from a list of elders provided by each band, from band employees (including band offices, schools, and businesses), and acquaintances. These subjects had never been diagnosed with diabetes mellitus or "borderline diabetes". Analysis of blood samples for indicators of metabolic control (glucose and fructosamine) further defined the two groups of subjects. One subject had hyperglycemia and high plasma fructosamine, and was therefore reclassified as diet controlled diabetes. Non-fasting glucose was 11.4 ± 5.2 mM (mean ± S.D.) and 6.1 ± 1.1 mM for the diabetic (n=60) and controls (n=50) groups, respectively, and fructosamine was 308.6 ± 80.5 μM and 227.6 ± 17.5 μM for the diabetic and controls groups, respectively. The study proposal was approved by the Human Ethics Committee of the Faculty of Home Economics, University of Alberta, and endorsed by the chief of each of the four First Nations in the study area, as well as the director of the Health Centre. The purpose and procedures were fully explained to each person individually in English or Cree, and written consent was obtained (Appendix 1). The final report was presented in written and oral form to each First Nation and the Health Center.

Interviews were conducted by two of the researchers and trained local assistants mainly at the health center, but also at people's homes and place of work. A total of 112

people participated in the study. Dietary and health information was provided by 110 persons (60 with and 50 without NIDDM) and complete information was provided by 92 persons. The 60 subjects represented 46 % of the 131 diabetic clients of the Health Center, who were eligible for our study. Another 40 persons with diabetes failed to keep appointments or refused to participate, while 31 could bot be contacted. The total number of participants per band was proportional to the band size (Samson was the largest, followed by Ermineskin, Lousis Bull, then Montana), and represented 10 % of the members 40 years and older. Plasma samples were obtained from 106 subjects (59 with and 47 without NIDDM).

Mean age of the subjects did not differ among the groups. Duration of diabetes was on average 9 to 10 years (Table 2.1). Those with diabetes were divided into three groups based on treatment of NIDDM. Groups treated with insulin, oral agents and diet alone had diabetes for an average of 12.5 ± 7.0, 6.6 ± 6.0 and 6.7 ± 7.0 years, respectively. The mean age of each group was similar, that is, 55.8 ± 11.3 , 56.6 ± 11.2, and 52.6 ± 11.4 years, for groups treated with insulin, oral agents, and diet alone, respectively.

The first interview included questions aimed at providing data of health particulars that may influence vitamin A status and control of diabetes (Appendix 2, Part D). Subjects with NIDDM and controls had similar age, dietary intake of vitamin A and zinc, body mass index, and health behaviors (Table 2.2). Drugs such as mineral oil, neomycin, cholestyramine and alcohol can affect vitamin A metabolism (Basu, 1988). No subjects reported use of the first three. Reported alcohol consumption in the past year was lower than expected. The 1991 aboriginal census data found that 70 % of respondents in Alberta age 15+ consumed alcohol in the past year (Statistics Canada, 1995). Subjects in this study were age 40 and older, and many indicated that they had stopped drinking alcohol because of its consequences. Our sample also had fewer smokers than expected.

Table 2.1. Number, age and duration of diabetes of study participants.

Gender and diabetes category	Number	Age (years)	Duration of diabetes (years)
Men			
With NIDDM	23	54.5 ± 11.2	10.1 ± 7.3
Controls	18	51.6 ± 9.7	
Women			
With NIDDM	40	55.6 ± 10.7	9.2 ± 7.3
Controls	31	54.2 ± 12.3	

Mean ± S.D.

Table 2.2. Health Information of Study Participants

	With NIDDM (n = 62) (% of subjects)	Controls (n = 49) (% of subjects)
Smokers	41.0	46.9
Alcohol consumption:		
Past week	6.6	6.1
Past month	8.2	12.2
Past year	11.4	14.3
> past year	73.8	67.3
Medication	54.1	40.8
Medication for hypertension	27.0	12.2
Supplementation[1]	20.6	26.5
Multivitamin	6.3	10.2
Vitamin A supplement[2]	6.3	4.1

Chi squared analysis used to test differences between groups. The groups were not significantly different for any of these variables ($p < 0.05$).

1. *Includes multivitamins, vitamin A, iron, B-complex, vitamin E, vitamin C, and other health food supplements such as bee pollin and lecithin.*
2. *Includes liver oil.*

The 1991 aboriginal census data reported that 59 % of respondents in Alberta age 15+ smoke daily or occasionally (Statistics Canada, 1995). The percentage of subjects who reported smoking, alcohol consumption, and use of supplements was similar among those with NIDDM compared to controls. A higher percentage of those with NIDDM reported use of medication for hypertension, although this was not significantly significant. Following diagnosis of diabetes, most subjects reported that they had made a dietary change (Table 2.3). About two-thirds had reduced their sugar and/or fat intake, while about one-third had reduced their total energy intake. Most followed a regular pattern with set times for meals and snacks. The majority had been instructed by a dietitian in a hospital setting.

In summary, this select sample of First Nation people live a modern lifestyle and are relatively prosperous. Their lifestyle no longer consists primarily of hunting and gathering food, although these may be occasional activities for some people. Band members live in houses with modern facilities, use trucks and cars for transportation, and consume mainly commercial foods. There is good access to health services nearby. Subjects with and without diabetes are similar in regards to average age and health status.

Table 2.3. Self-reported dietary changes made, nature of current diet, and source of diabetic instruction of participants with diabetes (n=60).

	Number	Percent
Made a dietary change after diagnosis	51	85
Nature of dietary change		
Reduced sugar intake	42	70
Reduced fat intake	36	60
Reduced energy intake	19	32
Reduced salt intake	7	12
Reduced alcohol intake	2	3
Current diet		
Eats the same number of meals and snacks each day	46	77
Eats at the same time each day	43	72
Was instructed to eat the same number of meals and snacks at the same time each day	43	72
Eats a specified number of calories each day	22	37
Source of diabetic instruction:		
Location		
Hospital	45	75
Medical clinic	8	13
Health center	8	13
Other	4	7
Instructor		
Dietitian	39	65
Doctor	14	23
Nurse	14	23
Self (reading)	2	3
Mother	1	2
Not instructed	1	2

Numbers sum to > 60, since some reported > 1 location and instructor.

Parameters Studied

This study was undertaken to determine if a select group of First Nation adults with NIDDM have impaired transport of vitamin A. The specific objectives of this study were to: 1) determine if Plains Cree adults with NIDDM have reduced vitamin A and RBP status compared to non-diabetic controls; 2) assess the influence of factors that may affect RBP status including nutritional status, metabolic control of diabetes and plasma insulin levels; and 3) determine the nutritional status of persons with diabetes and non-diabetic controls. The first and second objectives were examined by measuring plasma retinol and retinol binding protein (RBP), plasma zinc, plasma proteins (i.e., total protein, percent nitrogen, and albumin), metabolic control (i.e., glucose, fructosamine), and plasma insulin. Transthyretin, also necessary for the transport of retinol, was measured. Plasma cholesterol and triglyceride were measured to further describe the groups, and because lipoproteins may carry small amounts of retinol. α-Tocopherol was measured to confirm that the absorption of fat soluble vitamins was normal. Dietary intake of vitamin A was assessed by a vitamin A food frequency questionnaire. The third objective of the study was achieved by collecting anthropometric measures, (i.e., height, weight, waist, hip, and triceps skinfold) to assess long term nutrient status, and three 24-hr non-consecutive food recalls were obtained, to assess recent nutrient intakes.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Science (SPSS inc., 1993). The effect of gender and treatment for diabetes (namely, insulin, oral hypoglycemic agents, and diet alone) on variables was examined; subjects were pooled together if no effect was found. Each chapter's methodology contains a more detailed description of statistical analyses performed.

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3. ANTHROPOMETRIC CHARACTERISTICS

In recent decades, the prevalence of non-insulin dependent diabetes mellitus (NIDDM) has increased dramatically in the North American aboriginal population, and now for many Indian groups exceeds that of the general population (Young, 1990; Knowler et al., 1991; Young, 1993; Young, 1994). A high prevalence of obesity has also been documented among many North American aboriginal groups (Young, 1989; Broussard et al., 1991; Evers et al., 1989; Montour et al., 1989; Warne et al., 1993; Brassard et al., 1993; Santé Quebec, 1994; Szathmáry, 1994; Young and Harris, 1994). The association of obesity with NIDDM has long been recognized. Measures of adiposity have been associated with NIDDM within aboriginal groups, who vary greatly in prevalence of the disease and degree of acculturation (Evers et al., 1989; Montour et al., 1989; Knowler et al., 1991; Brassard et al., 1993; Warne et al., 1993; Szathmáry, 1994; Young and Harris, 1994).

Although the age-adjusted prevalence of diabetes mellitus (DM) in Alberta native people is approximately twice the Canadian population (5.1 % compared to 2.4 %) and is higher in females than males (Figure 1.1) (Young, 1990), to date there are no reports of anthropometric characteristics of Plains Cree in Alberta. The present work was undertaken to compare the anthropometric measures of a select sample of Plains Cree adults age 40 and older with and without NIDDM from Central Alberta. The objective of this paper is to confirm the significance of anthropometric characteristics in relation to diabetes within this group of aboriginal people.

Methods

The four First Nations that participated in the study, recruitment, consent of subjects, and ethics approval of the study have been previously described (Chapter 2.)

Anthropometric measurements were taken at the Health Centre by two of the

researchers and a trained local assistant (Appendix 2, Part E). Participants were weighed and measured in indoor clothing without shoes. Weight was measured to the nearest 0.5 kg, height to the nearest 0.5 cm, waist and hip circumference to the nearest 0.5 cm, mid upper arm circumference to 0.5 cm, and triceps skin fold using Lange calipers to the nearest 0.5 mm. Triceps skinfold was measured three times (or more) per participant, until measures agreed within 1 mm. Waist, hip and triceps measurements were preformed as described by Gibson (1990). The waist was measured from between the lowest rib and iliac crest to the umbilicus.

Body mass index (BMI) (wt/ht^2), a index of overall obesity, and waist:hip ratio (WHR), a index of upper body adiposity, were calculated. Means and the distribution were examined for groups with and without (controls) NIDDM, in relation to reference standards (Nutrition Canada, 1980; Jette, 1981).

Statistical analyses were performed using SPSS for Windows (SPSS Inc., 1993). Data from men and from women were analyzed separately. T-tests were used to identify differences between groups, and means and standard deviations were reported.

Results and Discussion

A total of 103 persons provided anthropometric data. Exclusions included others confined to a wheelchair, and those who declined to me measured. Numbers of men and women in diabetic and control groups, their mean age, and duration of diabetes were described elsewhere (Table 2.1).

Mean anthropometric values (Table 3.1) were within the range reported for other Canadian Indian groups with and without DM, in urban and remote areas (Evers et al., 1989; Montour et al., 1989; Brassard et al., 1993; Young and Harris; 1994).

Table 3.1. Anthropometric characteristics of persons with NIDDM and controls.

Measure	With NIDDM		Controls	
	n		n	
Men				
Weight (kg)	21	94.4 ± 20.3[1]	14	86.5 ± 16.0
Height (m)	21	1.75 ± 0.08	14	1.72 ± 0.07
Body Mass Index (BMI)	21	30.7 ± 6.3	14	29.2 ± 4.3
Waist circumference (cm)	17	114 ± 18	14	104 ± 12
Hip circumference (cm)	17	114 ± 17	14	106 ± 6
Waist hip ratio	17	1.00 ± 0.06	14	0.98 ± 0.06
Mid upper arm circumference (mm)	16	343 ± 63	14	343 ± 34
Triceps skinfold (mm)	16	20.8 ± 10.1	14	13.6 ± 4.3*
Women				
Weight (kg)	36	84 ± 14.1	32	79.9 ± 13.7
Height (m)	36	1.58 ± 0.06	32	1.59 ± 0.06
Body Mass Index (BMI)	36	33.6 ± 4.5	32	31.5 ± 4.8
Waist circumference (cm)	32	112 ± 13	32	105 ± 14
Hip circumference (cm)	32	120 ± 13	32	117 ± 11
Waist hip ratio	32	0.93 ± 0.07	32	0.90 ± 0.07*
Mid upper arm circumference (mm)	32	342 ± 47	29	330 ± 48
Triceps skinfold (mm)	33	28.6 ± 5.8	30	28.0 ± 7.2

1. mean ± S.D.

* *significantly different, $p < 0.05$, t-test*

Two measures, which included the triceps skinfold in men and waist:hip ratio in women, were significantly higher in those with NIDDM compared to controls ($p < 0.05$) (Table 3.1). Similarly, a cross-sectional study of an aboriginal group in south-western Ontario found greater central obesity, indicated by waist and hip circumferences, in those with DM, although other measures, including weights, heights, and triceps skinfolds were not

significantly different (Evers et al., 1989). In an incidence study of new cases appearing over time in Cree and Ojibwa people in northern Ontario and Manitoba, three indices, namely BMI, WHR, and subscapular/ triceps skinfold ratio, showed significantly larger values for new cases compared to controls (Young and Harris, 1994).

Mean values of groups provide an overall description for comparative purposes. Of greater concern from a health perspective is the proportion of persons at the upper extreme of the distribution, since these people may be at greatest risk for developing NIDDM. Compared to reference standards from large population groups, many adults in the present sample exceeded the 90th percentile for weight, mid upper arm circumference, and triceps skinfold (42 - 82 % depending on the measure) (Table 3.2). The percentage of persons exceeding the 95th percentile was usually higher in the group with NIDDM than controls.

A high prevalence of obesity was found in both the diabetic and control groups, as shown by BMI (Figure 3.1). Over 60 % of men with and without NIDDM had BMI's greater than 27, a value associated with increased risk of health problems (Bjorntorp, 1985; Health and Welfare Canada, 1988). Among women, 92 % with NIDDM and 75 % without NIDDM had a BMI greater than 27. This is similar to other groups with a high incidence of NIDDM (Montour et al., 1989; Brassard et al., 1993). In the Mohawk, 86 % of subjects with DM were obese, while 74 % without DM were obese (BMI > 27 for men and > 25 for women) (Montour et al, 1989). Among the James Bay Cree, 71 and 80 % of men and women with DM, respectively, had a BMI > 26 (Brassard et al, 1993). Although obesity was defined using different BMI values, all these studies showed a high prevalence of obesity. In the Cree and Ojibwa study, BMI was a strong independent predictor of DM (Young and Harris, 1994). Likewise, among the Pima Indians of Arizona, BMI was a strong risk factor for development of DM, although many other measures were equally predictive after controlling for age and gender (Warne et al, 1993).

Table 3.2. Percentage of persons with NIDDM and controls in reference percentile ranges for height[1], weight[1], mid-upper arm circumference (MUAC)[2], and triceps skinfold[2].

		Percentile range						
	n	<10th	<25th	<50th	<75th	<90th	<95th	>95th
Men:								
Height								
with NIDDM	21	0	10	14	24	10	5	38
controls	14	7	21	29	21	0	14	7
Weight								
with NIDDM	21	0	0	5	29	19	5	43
controls	14	7	0	14	14	57	0	7
MUAC								
with NIDDM	16	0	12	6	19	19	12	31
controls	14	0	0	7	21	19	21	21
Triceps skinfold								
with NIDDM	16	0	0	0	19	25	19	38
controls	14	0	7	21	29	29	7	7
Women:								
Height								
with NIDDM	36	8	17	25	14	22	8	6
controls	32	6	9	22	19	28	6	9
Weight								
with NIDDM	36	0	6	11	14	33	11	25
controls	32	0	6	22	16	34	16	6
MUAC								
with NIDDM	32	3	3	6	19	34	16	19
controls	29	0	10	14	28	14	17	17
Triceps skinfold								
with NIDDM	33	0	0	12	30	15	18	24
controls	30	0	3	10	27	23	17	20

1 *Reference standard - Nutrition Canada Anthropometry Report (1980). Height and weight for Canadian Indian population.*

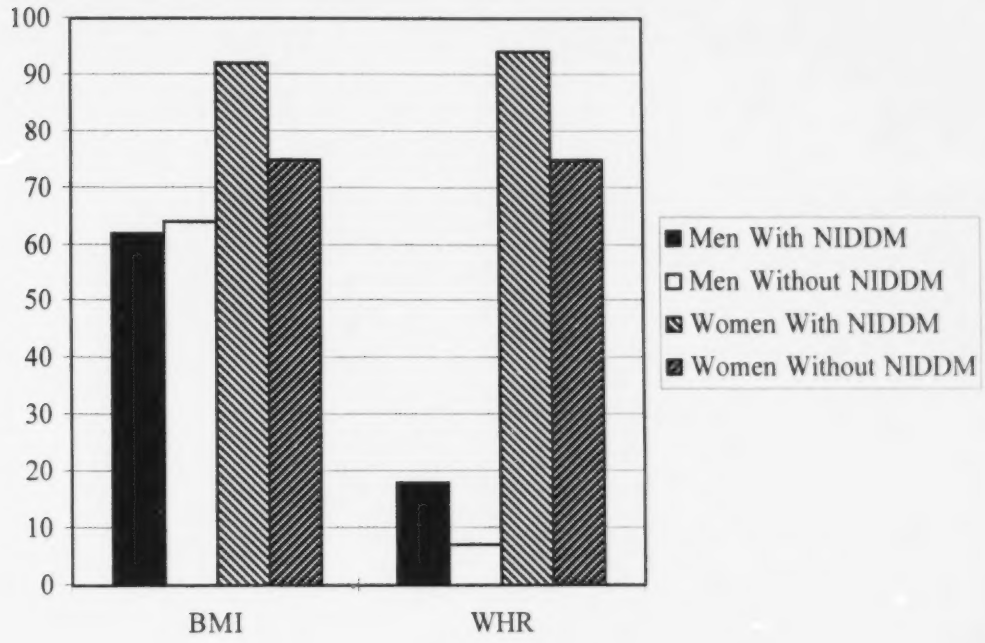
2 *Reference standard - Jette (1981).*

Central obesity, which is estimated by WHR, is a characteristic of the insulin

resistance syndrome (Reaven, 1988; Davidson, 1995). A WHR greater than 1.0 for men and 0.8 for women has been associated with increased risk of cardiovascular disease and related deaths (Bjortorp, 1985). More subjects with NIDDM than without, and more females than males, had a WHR that indicated risk (Figure 2.1). This is consistent with the theory that insulin resistance conferred a selective advantage for aboriginal groups during hunter/ gather times, but may now be the underlying metabolic characteristic predisposing them to obesity and NIDDM after adoption of a western lifestyle (Zimmet, 1990; O'Dea, 1991; Bogardus, 1993).

Regardless of the presence or absence of NIDDM, a large proportion of adults in this sample of Plains Cree in central Alberta are clearly at increased risk of health problems, due to excess body fat. In a population with a high prevalence of NIDDM, there is an urgent need to encourage the adoption of increased regular physical activity and more healthy eating patterns to reduce the magnitude and prevalence of obesity and its associated burden on health. It is especially important that community-driven programs directed at the younger generations be initiated to prevent the development of obesity. For those individuals who have developed NIDDM, treatment must focus on encouraging weight loss to enable metabolic control of NIDDM and prevent complications. Furthermore, many individuals in the group without NIDDM, as a result of their obesity, may be at risk of developing NIDDM in the future. Onset of NIDDM is often associated with few obvious symptoms; it is therefore important that physicians be encouraged to regularly screen for the presence of NIDDM among their patients.

Figure 3.1. Percent of persons with NIDDM and controls in categories of risk according to body mass index (BMI)[1] and waist hip ratio (WHR)[2].



1 Percentage of subjects with BMI > 27, calculated as wt/ht^2, where weight is in kg and height is in m.

2 Percentage of subjects with WHR > 1.0 for men and > 0.8 for women.

Although few studies have examined perceptions of DM among indigenous peoples (Tom-Orme, 1994; Garro and Lang, 1994; Joe, 1994), they provide useful insights for health educators and health care providers. For example, a large body size is considered desirable and healthy among Navijos and Utes of southwestern USA (Tom-Orme, 1994). A health knowledge survey among central Alberta Indian youth (including the four First Nations of this study) found that only 29 % of the grade 7-9 students who responded generally did not know that overweight people tend to be sick more often than people of normal weight (McKinnon et al., 1991). These two examples illustrate the challenge and the need for culturally sensitive ways in which to increase understanding of the link between obesity and chronic disease in populations, such as the Plains Cree.

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4. NUTRIENT INTAKES

Diabetes mellitus (DM) is a relatively new disease which has increased to epidemic proportions among many aboriginal groups (Zimmet, 1990; Knowler et al., 1981; Young, 1993; Young, 1994). Diabetes mellitus among North American aboriginal people is predominantly the non-insulin dependent type, NIDDM, which is strongly affected by lifestyle. Dietary changes, including the advent of an assured food supply and substitution of modern high fat and low fibre foods for traditional foods (Zimmet, 1990; Young, 1993; Young, 1994), and decreased physical activity together have led to obesity, which is associated with increased prevalence of NIDDM (Knowler et al., 1981). DM in turn alters carbohydrate, lipid and protein metabolism and appears to affect vitamin A (Basu et al., 1989) and zinc (Mooradian and Morley, 1987; Walter et al., 1991) status.

There are no contemporary nutritional status studies of Plains Cree Indians in Alberta. The diets of other Canadian First Nations people are often low in calcium, vitamins A, C, and D, iron, folate, and zinc (Johnston et al., 1977; Sevenhuysen and Bogert-O'Brien, 1987; Johnston and Schneider, 1988; Wein et al., 1991; Wein et al., 1993; Campbell et al., 1994; Moffatt, 1995; Wein, 1995). Aboriginal groups who consume substantial quantities of traditional foods are more likely to meet recommended intakes of many nutrients (Kuhnlein, 1984; Wein et al., 1991). The present study was undertaken to compare nutrient intakes in men and women, age 40 and older, with and without (controls) NIDDM. Nutrient intakes were also assessed in relation to recommendations for health.

Methods

The four First Nations that participated in the study, recruitment, consent of subjects, and ethics approval of the study have been previously described (Chapter 2).

Dietary information was collected by personal interview conducted by two university researchers and First Nation assistants trained by them, using three non-consecutive 24-hour recalls per person (Appendix 2, Part A). Interviews were conducted three days per week from September through April. Graduated three dimensional food models were used to estimate portion sizes. A vitamin A food frequency questionnaire was also administered on one occasion (Appendix 2, Part B). It asked how often each of 15 types of foods high in vitamin A was consumed in the past year, and the usual portion size, using the same food models. Most interviews were conducted at the Health Centre, although some were done in homes or work places.

Data were coded for analysis by the researchers. Daily nutrient intakes were computed using a program on SPSS for Windows (SPSS, 1993), which used data from the Canadian Nutrient File (Health and Welfare Canada, 1988) supplemented with published values for fibre, zinc, saturated, mono- and poly-unsaturated fatty acids, and vitamin D, as described in detail elsewhere (Wein et al., 1993). The three day average from the 24-hr recalls was used to represent each person's usual intake. Daily intakes did not include nutrients from vitamin/ mineral supplements. Use of any vitamin/ mineral or other supplement at least occasionally was reported by 13 diabetic and 13 control participants (Table 2.2). Of these, 8 subjects with NIDDM and 7 control subjects took supplements containing vitamin A.

Nutrient intakes were not significantly different within each gender for persons with NIDDM treated with insulin, oral hypoglycemic agents, or diet alone, and thus, all were pooled into one diabetic group for analysis. Since some nutrient intakes did not follow a normal distribution, median daily intakes were examined, along with means per megajoule (MJ). Medians are preferred over means for dietary intakes because they are not influenced by outliers with very high nutrient intakes. Nutrient density (mean per MJ) was examined because it reduces the variability among individuals. Non-parametric

tests (Mann-Whitney U test, or also called Wilcoxon rank sum test) were used to determine statistically significant differences between groups with and without NIDDM (Ferguson, 1981). This test converts a dataset into ranked values, and uses these to determine significant differences between two independent samples. Statistical analyses were performed by SPSS for Windows (SPSS, 1993).

The proportion of persons at risk of inadequate intakes was assessed using the probability method (Anderson et al., 1982; Gibson, 1990). Nutrient intakes were classified into six classes, and the number of individuals with intakes within each class range was determined. This number was then multiplied by a probability factor for risk of inadequate intake for each class, and the sum of all the classes was divided by the sample size to obtain the percent at risk of inadequate intakes for that nutrient.

A total of 56 specific foods from the vitamin A food frequency questionnaire was coded. Responses were first calculated to a weekly basis, and were then divided by seven to represent daily intake. Using these intakes along with the usual portion size, daily vitamin A was calculated from the food frequency data, using the same computer program as for the 24-hour recalls,

Results

Overall, 110 subjects provided a total of 300 recalls. Three recalls per person were obtained from 92 persons, two recalls per person from 6 persons, and one recall from 12 persons. Recalls represented intakes mainly on weekdays, but included two holidays (Thanksgiving and Easter) within the dataset. Numbers of men and women in diabetic and control groups, their mean age and duration of diabetes were described elsewhere (Table 2. 1). A summary of descriptive data of the subjects with NIDDM including self-reported dietary changes, the nature of the current diet, and source of diabetic instruction were also described (Table 2. 3)

Daily consumption of foods in 10 food groups is presented in Table 4.1. The greatest weight of food was in the miscellaneous group, which included tea, coffee, and soft drinks. The high moisture content of these beverages accounts for much of the weight. Diets of both those with and without NIDDM emphasized cereals and cereal products, followed by meats, poultry, fish and eggs. The amount of food by weight suggested that fruits were preferred over vegetables. Energy consumption from fruits and vegetables, however, appeared similar, and in fact high for vegetables among controls. This may reflect greater consumption of high energy vegetables such as French fries. Consumption of dairy products was low. If an adult consumed the recommended minimum of two servings of dairy products per day, at least 10 % of daily energy would come from dairy products (Health and Welfare, 1992). Compared to controls, those with NIDDM consumed a greater weight of dairy products, more energy from wild foods, and less energy from sweets ($p < 0.05$).

Table 4.1. Mean daily consumption by food group of persons with NIDDM and controls (in descending order as % of daily energy).

Food group	Grams of food		Energy (MJ)		% of daily energy	
	With NIDDM[1]	Controls[1]	With NIDDM[1]	Controls[1]	With NIDDM[1]	Controls[1]
Cereal	285	255	2.54	2.45	32	30
Meat, poultry, fish, eggs	166	175	1.72	1.93	21	24
Vegetables	208	168	0.66	0.78	8	10
Fruit	352	241	0.79	0.58	10	7
Misc.[2]	1228	1498	0.46	0.58	6	7
Dairy	201	110*	0.56	0.42	7	5
Fats	16	16	0.48	0.47	6	6
Wild food[2]	66	38	0.41	0.23*	5	3
Sweets	21	31	0.20	0.44*	3	5
Nuts	10	10	0.24	0.24	3	3

1. *n= 60 for those with NIDDM ; n= 50 those without NIDDM (controls) .*
2. *Includes tea, coffee, soft drinks, soups.*
3. *Includes wild game meat, birds, and berries.*

* *Statistically significant difference from preceding column, $p < 0.05$, Mann-Whitney U Test.*

Compared to population reference groups (Health and Welfare Canada, 1990), daily energy intakes appeared somewhat low. Percentage of energy from fat and saturated fat were higher than the recommended limit for all groups; however, women with NIDDM only slightly exceeded the recommended limit (Table 4.2) (Health and Welfare Canada, 1990). Dietary fibre intakes are recommended to be between 20 and 30 g/ day (Health and Welfare Canada, 1985). Median intakes were similar to the Canadian population and were about half the recommended level (Table 4.2). Compared to controls, men and women with NIDDM appeared to have higher dietary fiber consumption, and lower total fat, saturated fat, and sugar consumption.

Table 4.2. Median macronutrient intakes per day from food (excluding supplements) for persons with NIDDM and controls, and recommended nutrient intakes (RNI).

	Men		Women		
	With NIDDM[1]	Controls[1]	With NIDDM[1]	Controls[1]	RNI[2]
Energy (MJ)	8.5	8.4	6.2	6.6	
Protein, g (%)[3]	78 (16)	80 (15)	64 (16)	58 (15)	10 - 20 %
Total fat, g (%)[3]	76 (37)	88 (39)	50 (32)	67 (37)	< 30 %
SFA, g (%)[3,4]	25 (11)	31 (14)	19 (12)	23 (13)	< 10 %
MUFA, g (%)[3,4]	29 (13)	34 (15)	19 (12)	26 (15)	
PUFA, g (%)[3,4]	15 (17)	14 (6)	8 (5)	12 (7)	
Cholesterol, mg	445	397	213	242	< 300 mg
Carbohydrate, g (%)[3]	220 (43)	222 (44)	207 (54)	203 (51)	50 - 60 %
Starch, g	146	141	122	144	
Sugar, g	58	86	70	79	
Dietary fiber, g	15	13	15	12	

1. *n= 22 for men and n= 38 for women with NIDDM ; n= 17 for men and n= 33 for women without NIDDM (controls) .*
2. *Health and Welfare Canada, 1990; age 50 - 75.*
3. *Numbers in parentheses are percent of energy*
4. *Abbreviations: SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polyunsaturated fatty acids.*

Estimated median nutrient intakes of iron, thiamin, riboflavin, niacin, and vitamin C exceeded recommended nutrient intakes (RNI's) (Health and Welfare Canada, 1990) (Table 4.3). Among men and only women with NIDDM, median daily intakes of phosphorus exceeded the recommended nutrient intakes (RNI's). Median intakes of vitamin D, calcium, zinc, folate and vitamin A, based on the 24-hour food recalls, were below recommended levels for all groups, while median intakes of vitamin A, based on the food frequency method, were low only for control women. Vitamin A estimated from the food frequency questionnaire was higher than that estimated from three 24-hour recalls.

Table 4.3. Median micronutrient intakes per day and per MJ from food (excluding supplements) of persons with NIDDM and controls, and recommendated nutrient intakes (RNI).

	Men		Women		
	With NIDDM[1]	Controls[1]	With NIDDM[1]	Controls[1]	RNI[2] (men, women)
Calcium, mg	608	626	660	424	800, 800
Phosphorus, mg	1151	1131	982	796	1000, 850
Iron, mg	17	16	14	12	9, 8
Zinc, mg	11	9	8	8	12, 9
Vitamin A, RE[3]	772	690	660	491	1000, 800
Vitamin A, RE[4]	997	1012	912	784	1000, 800
Carotene, RE	258	261	279	181	
Thiamin, mg	1.6	1.4	1.4	1.2	0.9, 0.8
Riboflavin, mg	1.5	1.6	1.4	1.2	1.2, 1.0
Niacin, mg	20	20	16	16	16, 14
Folate, µg	201	156	164	125	230, 195
Vitamin C, mg	90	79	98	60	40, 30
Vitamin D, µg	2.5	2.5	3.3	1.8	5, 5

1. *n= 22 and n= 38 for men and women with NIDDM ; n= 17 and n= 33 for men and women without NIDDM (controls) .*
2. *Health and Welfare Canada, 1990; age 50 - 75.*
3. *Total daily vitamin A from 24-hr food recall method.*
4. *Total daily vitamin A per day from food frequency method.*

Diets of men with and without NIDDM showed few statistically significant differences in macronutrient intake (Table 4.4). Compared to control men, men with NIDDM had lower mean intake per megajoule (MJ) of sugar and greater mean intake per MJ of dietary fiber ($p < 0.05$). Compared to control women, women with NIDDM had lower intakes per MJ of total fat, mono- and polyunsaturated fatty acids, and higher intakes per MJ of protein, starch, dietary fibre ($p < 0.05$).

Table 4.4. Macronutrient intakes per MJ from food (excluding supplements) of persons with NIDDM and controls.

	Men		Women	
	With NIDDM[1]	Controls[1]	With NIDDM[1]	Controls[1]
Protein, g	10.3 ± 2.2	9.3 ± 1.2	10 ± 1.8	8.9 ± 1.8*
Total fat, g	9.8 ± 2.4	10.3 ± 1.6	8.5 ± 1.5	9.5 ± 1.8*
SFA[2], g	3.3 ± 1.0	3.8 ± 0.9	3.1 ± 0.7	3.1 ± 0.7
MUFA[2], g	3.8 ± 1.0	4.0 ± 0.7	3.2 ± 0.7	3.6 ± 0.8*
PUFA[2], g	1.8 ± 0.6	1.6 ± 0.3	1.3 ± 0.5	1.7 ± 0.6*
Cholesterol, mg	56 ± 24	52 ± 26	44 ± 24	39 ± 21
Carbohydrate, g	28 ± 5.6	28 ± 4.8	32 ± 5.0	30 ± 5.8
Starch, g	19 ± 5.7	17 ± 3.1	20 ± 3.4	17 4.6*
Sugar, g	7.3 ± 3.3	10.3 ± 3.6*	11 ± 4.3	12 ± 5.7
Dietary fiber, g	2.3 ± 2.3	1.6 ± 0.74*	2.5 ± 1.0	2.0 ± 1.4*

1. *n= 22 for men and n= 38 for women with NIDDM ; n= 17 for men and n= 33 for women without NIDDM (controls).*
2. *Abbreviations: SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polyunsaturated fatty acids.*

* *Statistically significantly different from preceding column, $p < 0.05$, Mann-Whitney U Test; values are mean ± S.D..*

Micronutrient intakes of men with NIDDM and controls were similar except that mean per MJ energy consumption of zinc and folate were greater for men with NIDDM ($p < 0.05$) (Table 4.5). Women with NIDDM consumed diets that were more nutrient dense than those of the controls. Intakes per MJ energy were greater for calcium, phosphorus, iron, vitamin A, thiamin, riboflavin, folate, vitamin C, and vitamin D ($p < 0.05$).

Table 4.5. Micronutrient intakes per MJ from food (excluding supplements) of perons with NIDDM and controls.

	Men		Women	
	With NIDDM[1]	Controls[1]	With NIDDM[1]	Controls[1]
Calcium, mg	75 ± 26	70 ± 19	104 ± 34	70 ± 32*
Phosphorus, mg	146 ± 27	133 ± 21	159 ± 35	136 ± 61*
Iron, mg	2.1 ± 0.56	1.9 ± 0.34	2.2 ± 2.15	1.9 ± 0.56*
Zinc, mg	1.3 ± 0.38	1.1 ± 0.27*	1.2 ± 0.33	1.2 ± 0.36
Vitamin A, RE[2]	118 ± 124	80 ± 32	208 ± 472	103 ± 180*
Thiamin, mg	0.21 ± 0.059	0.17 ± 0.046	0.22 ± 0.048	0.19 ± 0.089*
Riboflavin, mg	0.22 ± 0.070	0.20 ± 0.066	0.25 ± 0.13	0.19 ± 0.07*
Niacin, mg	2.8 ± 0.75	2.5 ± 0.49	2.6 ± 0.55	2.5 ± 0.71
Folate, µg	24 ± 6.0	19 ± 6.1*	27 ± 12.4	20 ± 7.5*
Vitamin C, mg	15 ± 11	9 ± 6	21 ± 16.4	12 ± 7.6*
Vitamin D, µg	0.43 ± 0.26	0.30 ± 0.18	0.56 ± 0.33	0.37 ± 0.28*

1. *n=22 and n=38 for men and women with NIDDM ; n=17 and n=33 for men and women without NIDDM (controls) .*
2. *Total daily vitamin A from 24-hr food recall method.*

* *Statistically significantly different from preceding column, $p < 0.05$, Mann-Whitney U Test; values are mean ± S.D.*

More than 50% of participants were at risk of inadequate intakes of vitamin A (by the 24-hour recall method), vitamin D, calcium, and folate (Table 4.6). However, the proportion at risk of inadequate vitamin A intakes was lower when probability assessment was based on food frequency data. More than 40% were at risk for inadequate zinc intakes. Compared to controls, fewer persons with NIDDM were at risk for most nutrients. In general, more women than men were at risk.

Table 4.6. Percentage of persons at risk of inadequate nutrient intakes[1].

Nutrient	Entire sample	With diabetes	Controls	Male	Female
n	110	60	50	39	71
Vitamin D	55	49	63	56	55
Vitamin A[2]	53	44	64	47	54
Vitamin A[3]	27	25	29	30	22
Calcium	53	43	60	55	52
Folate	51	40	63	49	52
Zinc	40	35	45	39	40
Phosphorus	19	16	23	16	20
Protein	13	12	15	10	15
Niacin[4]	11	11	11	8	13
Vitamin C	10	4	16	12	8
Riboflavin	9	8	11	8	14
Iron	8	5	11	2	11
Thiamin	4	2	7	4	5

1. *Probability method of Anderson et al (1982) as described by Gibson (1990).*
2. *Total daily vitamin A from 24-hr food recall method.*
3. *Total daily vitamin A per day from food frequency method.*
4. *May be too high, since calculated from preformed naicin alone, without allowing for conversion from tryptophan.*

Discussion

Three recalls per person were averaged to represent usual intake and to minimize day-to-day variation within an individual's diet. The 24-hour recall method is quick and does not require skill in written English (Gibson, 1990). It is also a culturally sensitive instrument, which by requiring recall of everything eaten the previous day, cannot be thrown off by cultural variations in meal patterns or meanings of words (Cassidy, 1994). Individuals eat differently on a day by day basis, and this intraindividual variance may result in misclassification of subjects in regards to estimates of adequacy of intake

(Beaton et al., 1983). For nutrients that are found in many different foods (ie., energy and macronutrients) there is less intraindividual variation. However, for nutrients such as cholesterol, polyunsaturated fatty acids, and vitamin A, intraindividual variance increases, and thus, these estimates of usual intake must be viewed with caution (Beaton et al., 1979; Beaton et al., 1983). Although for logistic reasons the recalls cover mainly weekdays, two holiday Mondays within the dataset ensured that a few special meals were included. First Nation people of central Alberta now rely primarily on commercial foods (rather than wild foods) (Table 4.1); hence, seasonal differences between September and April were not expected nor studied.

To accurately reflect long term usual intake of an individual (as opposed to a group), 7-10 recalls per person are required for most micronutrients (Block and Hartman, 1989). For nutrients with high day-to-day variability such as vitamin A, even more recalls are required (Beaton et al., 1983). Since vitamin A tends to be concentrated in certain foods which may not be obtained regularly, a vitamin A food frequency questionnaire was also used. It covered a longer time frame than the 24-hour recalls, and included foods consumed in the past year resulting in higher estimates of vitamin A consumption. For example, foods consumed perhaps only once a year, such as pumpkin pie at Thanks-giving, were included. Some fresh foods such a Swiss chard and fresh peaches were consumed only during the summer, and hence appeared only in the food frequency data. The food frequency method, however, may have overestimated vitamin A intakes. The interviewer asked about specific foods, which may have given the impression that these were desirable foods, introducing a potential source of bias. Due to the limitations of each method, the vitamin A intakes reported here should be interpreted with caution. It is likely that the true vitamin A intakes of this sample are higher than they appear from the 24-hour recalls, but may not be as high as suggested from the food frequency approach. Further work is needed to develop and validate within this

population group a better food frequency instrument for estimating dietary vitamin A and carotene.

Others have recommended using a food frequency method for estimating vitamin A intakes. In a comparison study of three dietary methods for estimating vitamin A, the food frequency questionnaire produced the highest mean daily estimate, compared to a single 24-hour recall and a three-day food record, and the researchers concluded that the food frequency method is the preferable method of estimating vitamin A intake (Russell-Briefel et al., 1985). Gray et al (1984) reported that the food frequency method provided good estimates of vitamin A intakes for groups, but less useful estimates for individuals. Hankin (1987) recommended a specific quantitative diet history method (similar to a quantitative food frequency) which had been developed and validated among an Hawaiian population, for estimating dietary vitamin A and carotene. Her instrument covered 85 % of food sources of vitamin A used by that population, allowed variable portion sizes, covered a long time period, was objective, and had been examined for validity and reproducibility. Validation of dietary assessment methods in other aboriginal populations is needed (Hankin and Wilkens, 1994).

Nutrient Intakes

Energy intakes were generally lower than expected based on anthropometric data; however, the latter data (Chapter 3) reflect long-term intake rather than current intake. Current intakes may have been underreported. Some persons may have had difficulty remembering all the food items consumed, although persons with obvious memory problems were excluded during recruitment. Additionally, some persons may have underestimated quantities of foods or failed to report foods they felt they should not have consumed, such as fat and alcohol.

The food consumption data suggest that women obtain a greater proportion of

daily energy from fruits, cereals, and vegetables, while men consume more meat, dairy products, and fats. Furthermore, differences in nutrient density between diets of persons with and without NIDDM were more pronounced among women than men. The majority of those with NIDDM reported a reduction in fat and sugar intake since diagnosis of NIDDM (Table 2.3), and this was reflected in significantly reduced mean per MJ consumption of these nutrients compared to controls.

Comparison to Other First Nation People

Macronutrient intakes were similar to those in other Canadian First Nation communities (Johnston et al., 1977; Wein et al., 1991; Wein et al., 1993); however, men had energy and protein intakes which were lower than reported for men near Wood Buffalo National Park (Wein et al., 1991), while women had higher energy intakes than in northern Alberta (Wein et al., 1993) or northern Manitoba (Campbell et al., 1994). The high risk of inadequate intakes of vitamins A and D, calcium, and folate is similar to other studies (Wein et al., 1991; Wein et al., 1993; Campbell et al, 1994; Wein, 1995).

Diets of Pima Indians of the American southwest (a group with extremely high prevalence of diabetes) have been extensively studied (Reid et al., 1971; Smith et al., 1991; Boyce and Swinburn, 1993). Baseline dietary data of Pima Indian women, aged 25 to 44, found that the average Pima diet was similar to the average U.S. diet, as estimated from food purchase data. Energy consumption was similar between those with and without DM, although women with DM reported lower sugar consumption (Reid et al., 1971). The 187 women who did not have DM at the time of the dietary survey were followed during the next decade, and 87 developed DM. The incidence of DM was related to total energy and carbohydrate intake at the time of the survey, but not sugar intake. Although fat consumption did not correlate with incidence of DM, it is an important contributor to total energy intake and was strongly related to total

carbohydrate intake (Bennet et al., 1984).

The aborigines in Australia retain knowledge of their traditional hunter-gatherer lifestyle, and have provided an opportunity to study the effect of a temporary reversion to a traditional lifestyle on the metabolic control of DM. A traditional lifestyle for 7 weeks resulted in weight loss, reduction in fasting glucose, hypertriglyceridemia, blood pressure, and improvements in glucose tolerance (O'Dea, 1991). A study of the effect of bush living in a Cree community in northern Quebec found only small improvements in fasting glucose and body weight after 3 months, which may be partly due to the quantity of commercial food consumed in the bush and greater energy consumption than in O'Dea's study (Robinson et al., 1991).

Among Canadian aboriginals, only one intervention study of nine Blackfoot women elders in southern Alberta examined nutrient intakes and found no differences between women with and without DM (Johnston and Schneider, 1988). Szathmáry et al (1987, 1994) examined many factors including macronutrient intakes and glucose tolerance among Dogrib Indian people of the Northwest Territories, a group still engaged primarily in a hunting-trapping lifestyle, and among whom DM was unknown when the study began in 1979. Among four Dogrib settlements with varying degrees of acculturation, the traditional food base was stable, but with acculturation new foods were added to the diet, instead of replacing traditional foods, resulting in greater total dietary energy (Szathmáry et al., 1987). The impact of dietary change on plasma glucose was not clear; however, over several years, a few cases of DM developed in the most acculturated village (Szathmáry, 1994).

Identifying the effect of dietary changes on the development of NIDDM is difficult since in acculturated communities, where DM is already a problem, dietary changes have already occurred. Longitudinal studies are necessary to identify causal relationships, and even these studies are unable to separate the influence of specific

nutrients since their consumption is so interrelated. However, at both extremes of high prevalence of DM among the Pima and very low prevalence among the Dogrib, the role of diet, especially excess dietary energy, has been implicated. Longitudinal studies of various aboriginal groups in relation to prevalence of DM, dietary change and nutritional status are needed to better understand the role of diet in the development of NIDDM among First Nations people.

In conclusion, this study found that persons with diabetes, especially women, consumed higher nutrient quality diets than control persons within the same First Nation community. It is apparent that persons with NIDDM have learned components of a healthier dietary pattern, and should be encouraged to pass on their knowledge to other community members. Dietary changes should be encouraged by both health professionals and community members to reduce the likelihood of inadequate nutrient intakes and to promote healthy body weights. Specifically, consumption of dairy products, fruits and vegetables, and a reduction in fats, should be encouraged. Nutrition and diabetes education needs to be directed to all community members, and especially children, to reduce the risk factors for development of this disease and prevent complications in those who have already developed it.

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5. STATUS OF RETINOL AND ITS CARRIER PROTEINS

Metabolic availability of vitamin A has been found to be impaired in individuals with insulin dependent diabetes mellitus (IDDM), as indicated by reduced levels of plasma retinol (Wako et al., 1986; Basu et al., 1989; Kempf et al., 1991; Martinoli et al., 1993) and retinol binding protein (RBP) (Basu et al., 1989; Kemp and Frindik, 1991). The reduced circulatory levels of retinol and its carrier proteins have also been demonstrated in streptozotocin-induced diabetic rats (Basu et al., 1990; Tuitoek et al., 1996a). While the plasma concentration of retinol is decreased, its hepatic level is increased in these diabetic animals (Basu et al., 1990; Tuitoek et al., 1996a; Leichter et al., 1991). The latter also had a decreased concentration of 11-cis-retinal (a constituent of rhodopsin) in the retina of the eye (Tuitoek et al., 1996a) These results suggest that the transport of retinol to its target tissues may be impaired in IDDM.

Only a few studies have examined vitamin A status of those with non-insulin dependent diabetes mellitus (NIDDM). Results of these studies, however, do not agree with the results reported from subjects with IDDM (Havivi et al., 1991; Straub et al., 1993). Thus, vitamin A status appears to be normal in NIDDM. Nonetheless, these studies determined neither vitamin A carrier proteins nor did they relate vitamin A status to the availability of insulin.

Non-insulin dependent diabetes mellitus has become a serious health problem for indigenous peoples around the world as its prevalence continues to increase in epidemic proportions. There are several reviews of the epidemiology and etiology of NIDDM in indigenous groups in Canada (Young et al., 1992; Young, 1993; Szathmáry, 1994), the United States (Gohdes et al., 1993) and the South Pacific (Zimmet et al., 1990). Indigenous people with NIDDM have frequent and serious diabetic complications, and mortality from diabetes among this population is greater than among the general population (Young, 1993; Gohdes et al, 1993; Zimmet et al., 1990)

The present study was undertaken to examine vitamin A status of a select group of Plains Cree adults with NIDDM, residing in Alberta.

Materials

The four First Nations (Indian bands) that participated in the study, recruitment, consent of subjects, and ethics approval of the study have been described (Chapter 2).

Sample Collection

Non-fasting blood samples were obtained from 59 subjects with NIDDM (38 females and 21 males) and 47 control subjects (30 females and 17 males). Blood samples were collected and separated plasma was stored at -40 °C for further analysis. These were used to measure plasma retinol, and the factors that influence its levels such as RBP, transthyretin (TTR), protein and α-tocopherol. In addition, glucose, fructosamine, insulin, and plasma lipids were measured to assess metabolic control of diabetes. Plasma for retinol analysis was kept in a separate container in the dark until analysis. Subject identification codes were written on tubes, and analysis was performed in a blind manner.

Analytical Methods

Plasma Retinol and α-Tocopherol Analysis. Retinol and α-tocopherol were analyzed simultaneously by high-performance liquid chromatography (HPLC) using a modification of the method by Nierenberg and Lester (1985). This method was improved by measuring absorbency at 300 nm instead of 280 nm. This increased the minimum detectable limit of retinol. Changing the wavelength to 300 nm excluded α-tocopherol acetate as an internal standard, and therefore, retinol acetate was used. The percent recoveries of retinol and α-tocopherol after 1 hour incubation in plasma were 95.1 ± 5.9

and 98.7 ± 3.7, respectively (Nierenberg and Lester, 1985). Same day coeffeicients of variation were less than 5 % for both retinol and α-tocopherol.

Analysis was performed using a Varian 5000 liquid chromatograph along with a Shimadzu Sil-9A auto injector (Columbia, MD), a Waters 486 turnable absorbance detector (Millipore, Mississauga, ON), and a Supelcosil LC-18 (15.0 cm x 4.6 mm) (Supelco, Mississauga, ON) reverse phase column with 3 μm packing. The gradient for the mobile phase was as follows: 95:5 (acetonitrile: methanol) at time 0, 0:100 at 4 minutes, and 95:5 at 8.5 minutes. Flow-rate was 1 mL/ min and injection volume was 100 μL. Plasma retinol and α-tocopherol concentrations were determined by the internal standard curve method, using peak areas (Nierenberg and Lester, 1985). Calculations were done using Ezchrom chromatography data system version 2.1.

Extraction of retinol and α-tocopherol was performed using a solvent extraction method as previously described (Nierenberg and Lester, 1985). Stock solutions of all-*trans* retinol and α-tocopherol were prepared in ethanol, and working standard dilutions were prepared in acetonitrile. Duplicate 200 μL plasma samples were mixed with 100 μL of acetonitrile containing 250 ng of retinol acetate (internal standard). Standards were prepared daily in the concentration ranges: 2.5 mg/L to 12.5 mg/L and 30 mg/L to 150 mg/L, for retinol and α-tocopherol, respectively. Standards were exposed to the same extraction procedure as samples. A mixture of retinol and α-tocopherol in acetonitrile, along with the internal standard, was added to 250 μl of deionized water and then extracted. All handling of samples and standards were performed in dim light and tubes were wrapped with aluminum foil. All solvents used were HPLC grade. All-*trans*-retinol, α-tocopherol, and all-*trans*-retinol acetate were obtained from Sigma (St. Louis, MO).

Plasma Zinc Analysis. Plasma zinc was determined by flame atomic absorption spectrophotometry (Perry, 1990) using a Perkin-Elmer 4000 atomic absorption spectrophotometer and burner control. Zinc standards (Fisher Scientific Ltd., Edmonton, Canada) and samples (0.40 mL) were diluted with 4.0 mL of a 30 % solution of Brij 35 (polyoxyethylene 23 lauryl ether) (Sigma, St. Louis, MO). Standards were aspirated after approximately every 10 samples and standard curves were prepared. All glassware was rinsed with a 20 % nitric acid solution and deinonized water, and samples were prepared in disposable polypropylene tubes with caps to prevent zinc contamination.

Plasma Proteins Analyses. Percent nitrogen was measured with a nitrogen analyzer (Leco FP-428, St. Joseph, MI); samples were prepared by drying 200 µL of plasma at 120 °C for 4.5 hours in a tin capsule. A Spectronic 3000 Array Spectrophotometer (Milton Roy), with Milton Roy Rapid Scan software, was used to measure UV absorbency for the following colormetric methods. Total plasma protein was determined by the Biuret method (Kaplan and Szabo, 1983) and standard curves were prepared using Behring Standard Human Serum (Hoeschst-Roussel Inc., Montreal, Canada). Plasma albumin was measured using a Stanbio Albumin Colormetric Test (Fisher Scientific Ltd., Edmonton, Canada) which is based on the bromocresol green method (Doumas, 1971). Behring Standard Human Serum (Hoeschst-Roussel Inc., Montreal, Canada) was used as an external control. Triglycerides and cholesterol were measured using Sigma kits and calibrators (Sigma Diagnostics, St. Louis, MO).

Plasma RBP and TTR levels were measured by radial immunodiffusion using Behring LC-Partigen Immunodiffusion Plates (Hoeschst-Roussel Inc., Montreal, Canada). Behring Standard Human Serum (Hoeschst-Roussel Inc., Montreal, Canada) was used to prepare a standard curve. The diameter of rings were measured after 72 hours at room temperature using a measuring viewer (Behring Diagnostics) to an accuracy of 0.1 mm and compared with standards. Behring Control Plasma for Partigen (Hoeschst-Roussel Inc.,

Montreal, Canada) was used to check accuracy.

Plasma Insulin, Glucose and Fructosamine Analyses. Insulin was measured with a Pharmacia Insulin RIA 100 radioimmunoassay (Pharmacia Co., Uppsala, Sweden). Glucose was determined using a glucose analyzer (Glucose Analyzer 2, Beckman Instruments Inc., Fullerton, CA). Fructosamine was measured with a Fructosamine kit (Cefalu, 1991) and Cobas-Bio device (Roche, Canada).

Data Analysis

Statistical analyses were performed by SPSS for Windows (SPSS Inc., 1993). Subjects with NIDDM were first compared to controls. Means and standard deviations (S.D.) were calculated and differences between means were assessed by two-way analysis of variance (ANOVA) which included the effect of gender. Gender did not have a significant effect on any of the parameters measured. Secondly, subjects with diabetes were pooled over gender and classified according to treatment for diabetes, namely insulin, oral agents, or diet alone, and the means of the parameters were compared with controls. Differences between the means were assessed by the Scheffé test. Multiple linear regression was used to identify which variables were important predictors of plasma RBP. The strength of association between pairs of variables was assessed by Pearson correlation coefficients.

Results

Mean insulin concentration was higher for subjects with NIDDM, although this did not reach statistical significance (Table 5.1). Non-fasting insulin measurements may be influenced by many factors including time of the last meal, medication received for diabetes treatment, and recent activity, and therefore cannot be used to assess insulin resistance. They do, however, indicate that the subjects with NIDDM were either still

able to produce insulin or were taking insulin injections. Insulin concentration was highest in subjects who reported receiving insulin injections ($p < 0.05$) (Figure 5.1). Mean plasma triglyceride concentrations for subjects with NIDDM were 1.4 times mean concentration for those without diabetes, although cholesterol concentrations were similar (Table 5.1). Metabolic control of diabetes was poor for many and subjects receiving insulin treatment had the highest concentrations of glucose and fructosamine ($p < 0.05$) (Figure 5.1).

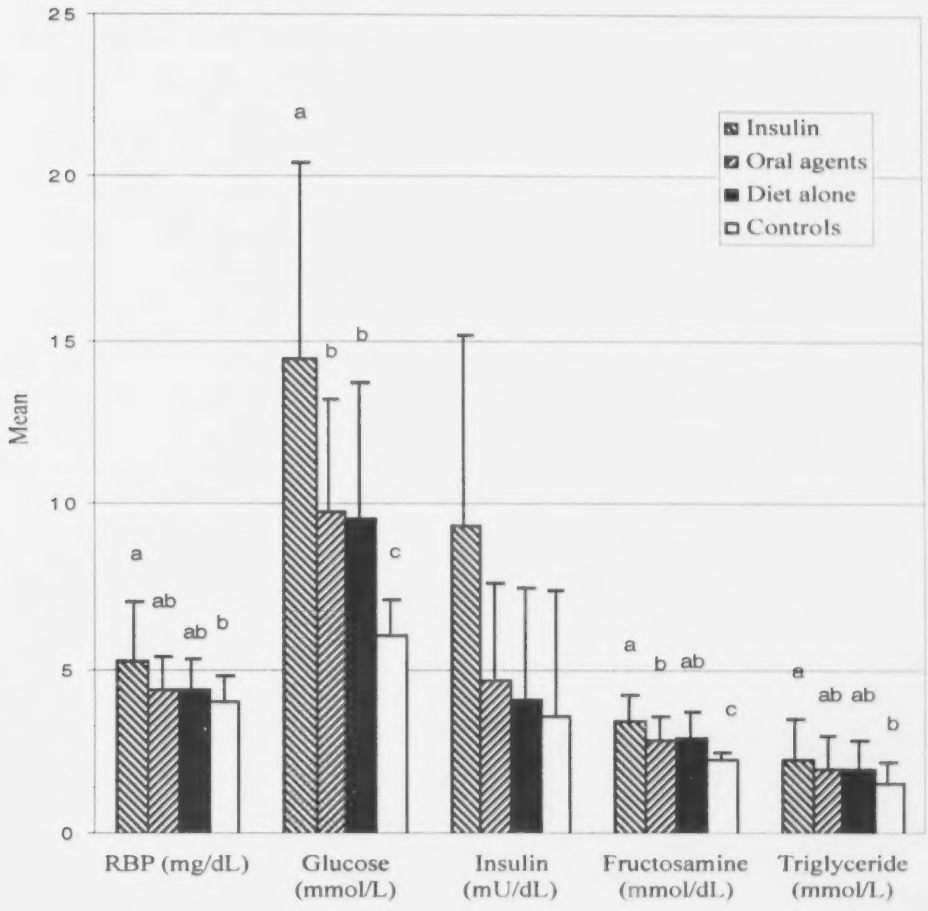
Table 5.1. Indicators of metabolic control for persons with NIDDM and controls.

Parameter	With NIDDM	Controls
Non-fasting glucose (mM)	11.4 ± 5.2 [1]***	6.1 ± 1.1
Fructosamine (μM)	308.6 ± 80.5***	227.6 ± 17.5
Triglyceride (mM)	2.11 ± 1.06**	1.53 ± 0.63
Cholesterol (mM)	4.82 ± 1.78	4.87 ± 1.45
Nonfasting insulin (μU/mL)	50.6 ± 40.0	36.4 ± 38.4

1. Values are mean ± S.D.

*Main effect of diabetes, controlling for gender, from two-way ANOVA. No significant interactions were found. F-test for differences between mean scores of groups; ** $p < 0.01$; *** $p < 0.001$*

Figure 5.1. Plasma concentrations of retinol binding protein and indicators of metabolic control in persons treated with insulin, oral agents, diet alone, and controls.



a,b,c- Bars with different letters are statistically significant; $p < 0.05$ Scheffé test. Error bars are S.D. Number of subjects in each group: insulin (n = 23); oral agents (n = 21); diet alone (n=17); control (n = 49).

Plasma retinol, tocopherol and zinc concentrations did not differ between subjects with NIDDM and controls (Table 5.2). The ratio of retinol to RBP was significantly lower in subjects with NIDDM ($p < 0.05$), which was likely a result of their higher retinol binding protein concentrations, without concomitantly higher retinol concentrations. Subjects receiving insulin had the highest concentration of RBP ($p < 0.01$) (Figure 5.1). Subjects with NIDDM had similar percentages of nitrogen and concentrations of total protein, albumin, globulin, and TTR compared to controls (Table 5.2).

Table 5.2. Plasma concentrations of retinol, α-tocopherol, zinc and proteins for persons with NIDDM and controls.

Parameter	With NIDDM (n=59)	Controls (n=47)
Retinol (μM)	2.23 ± 0.72[1]	2.14 ± 0.65
Retinol/ RBP	1.01 ± 0.25*	1.14 ± 0.36
∝-Tocopherol (μM)	25.9 ± 15.7	27.9 ± 16.3
Zinc (μM)	12.9 ± 1.8	13.3 ± 1.8
Total protein (g/L)	84.4 ± 16.3	84.2 ± 11.9
Nitrogen (%)	1.26 ± 0.10	1.28 ± 0.07
Albumin (g/L)	53.7 ± 6.7	53.7 ± 5.5
fix-Globulin (g/L)	30.4 ± 2.0	30.5 ± 1.6
RBP (mg/dL)	4.69 ± 1.41**	4.04 ± 0.79
TTR (mg/dL)	23.6 ± 4.8	24.2 ± 5.0

1. Values are mean ± S.D.

*Main effect of diabetes, controlling for gender, from two-way ANOVA. No significant interactions were found. F-test for differences between mean scores of groups; * $p < 0.05$; ** $p < 0.01$.*

Multiple regression analysis indicated that plasma retinol and TTR concentrations are the most important predictors of RBP concentration (Table 5.3). Plasma glucose was a predictor of RBP in subjects with NIDDM, but was not significant for controls. Number of years since diagnosis, and globulin concentrations were also significant predictors of RBP in subjects with NIDDM. Total protein, albumin, and tocopherol were predictors of RBP for controls.

Table 5.3. Multiple linear regression of plasma proteins, metabolic control, retinol, zinc, and duration of diabetes as predictors of plasma retinol binding protein concentration.

Predictor variable	Regression coefficient (mean ± SEM)	
	With NIDDM (n=51)	Controls (n=46)
Retinol (μM)	1.01 ± 0.15****	0.71 ± 0.17***
Transthyretin (mg/dL)	0.086 ± 0.023***	0.050 ± 0.024*
Total protein (g/L)	NS	0.017 ± 0.007*
Albumin (g/L)	NS	-0.059 ± 0.016**
∝-Tocopherol (μM)	NS	-0.021 ± 0.007**
Globulin (g/L)	0.018 ± 0.007*	NS
Glucose (mM)	0.056 ± 0.021*	NS
Years since diagnosis	0.040 ± 0.051*	N/A
Constant	-1.14 ± 0.57	3.61 ± 0.90***
Adjusted R^2	0.74	0.51

*Forward linear regression used to develop models; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.*
NS- not significant; N/A- not applicable

Discussion

Human subjects with IDDM and rats with streptozotocin-induced diabetic rats have been reported to have depressed concentrations of plasma retinol, as well as of its carrier proteins, including RBP and TTR (Wako et al., 1986; Basu et al., 1989; Martinoli et al., 1993; Kemp and Frindik, 1991; Basu et al., 1990; Tuitoek et al., 1996a). Reduced circulatory retinol was accompanied by a decreased level in the retina of the eye and an elevated level in the liver compared to the non-diabetic control rats (Tuitoek et al., 1996a). The transfer of vitamin A to its target site, thus, appears to be affected in the presence of IDDM or experimentally induced diabetes. In contrast, earlier studies have shown that the vitamin A status is not affected in subjects with NIDDM (Havivi et al., 1991; Straub et al., 1993). Using a select group of First Nation peoples, the present study has revealed results that support these earlier studies.

It is possible that the normal metabolic availability of vitamin A in these subjects may be adequate insulin levels to promote normal retinol mobilization. Non-insulin dependent diabetes mellitus develops when the β-cells in the pancreas fail to release enough insulin to compensate for the insulin resistance (Olefesky and Nolan, 1995). Insulin resistance is associated with a syndrome characterized by reduced insulin sensitivity, hyperinsulinemia, central obesity, hypertension, and hypertriglyceridemia without hypercholesterolemia (Davidson, 1995). This combination was initially called "Syndrome X" (Reaven, 1988) and has been more recently termed the "Insulin Resistance Syndrome" (Defronzo and Ferrannini, 1971; Davidson, 1995). Insulin resistance and hyperinsulinemia have been demonstrated to be part of the etiology of NIDDM in indigenous groups world-wide including the Pima Indians of Arizona (Bogardus, 1993), the Australian Aborigines (O'Dea, 1991), and the indigenous people from the South Pacific (Zimmet, 1990). Individuals with NIDDM may actually have high blood insulin

levels, a consequence of reduced insulin sensitivity or insulin resistance (Olefesky and Nolan, 1995).

In the present study, subjects with NIDDM exhibited characteristics of the Insulin Resistance Syndrome. It is possible that the normal metabolic availability of vitamin A in these subjects may be caused by insulin sufficiency (i.e., sufficient insulin to promote normal protein metabolism). Insulin may stimulate RBP synthesis and hence retinol release from the liver. Streptozotocin-induced diabetic rats have demonstrated that insulin administration can normalize plasma and liver vitamin A levels (Leichter et al., 1991; Tuitoek et al., 1996b).

Elevated levels of RBP were found in subjects with NIDDM and the highest levels in those receiving insulin treatment. Although insulin resistance was not measured, non-fasting insulin measurements suggested that the subjects with NIDDM had similar or higher insulin levels than controls. Retinol binding protein was associated with indicators of the insulin resistance syndrome (i.e., metabolic control, hyperlipidemia, and hypertension) in subjects with NIDDM, but not in controls. For subjects with NIDDM, RBP correlated with glucose ($r = 0.39$, $p < 0.01$), triglycerides ($r = 0.36$, $p < 0.01$), and use of hypertensive medications ($r = 0.48$, $p < 0.001$). Retinol binding protein did not correlate with insulin, which is not surprising since insulin measurements had high variance.

These results suggest that insulin resistance did not inhibit protein synthesis by the liver. This is in agreement with other studies that have found normal protein metabolism in subjects with NIDDM and normoglycemia (Staten et al., 1986; Welle and Nair, 1990; Biolo et al., 1992; Luzi et al., 1993; Pijl et al., 1994). Moreover, increased protein turnover has been associated with poor metabolic control of NIDDM (Gougeon et al., 1994). This may help to explain why the highest levels of RBP were found in the subjects with hyperglycemia. Hyperglycemia may be promoting insulin secretion in

those with partial β-cell function. Similar levels of albumin and TTR were found between subjects with NIDDM and controls. Insulin may exert a greater effect on RBP synthesis than albumin and TTR, since it has a short half-life of only 12 hours. Albumin and TTR have half-lives of 14- 20 days and 2-3 days, respectively.

Retinol binding protein may be affected by numerous parameters, including vitamin A and zinc status, trauma, protein status, age, and gender (Rask, 1980). Multiple linear regression was used to determine which of the variables measured were important predictors of plasma RBP levels. Plasma retinol and TTR were the most important predictors of RBP in both subjects with NIDDM and controls. Metabolic control, based on glucose, was a predictor of RBP in subjects with NIDDM but not in controls. This provides further evidence of the association between RBP and metabolic control of diabetes. Duration of diabetes was also a predictor of RBP in NIDDM. Endogenous insulin secretion may diminish with increased duration of diabetes, resulting in a need for insulin administration. Exogenous insulin may also be promoting RBP synthesis, since the insulin treated group had the highest RBP concentration. Predictors of RBP levels within the diabetic treatment groups could not be tested for as a result of inadequate sample size.

Retinol binding protein was significanly less saturated with retinol in subjects with NIDDM than controls, although plasma retinol did significantly correlate with RBP for subjects with NIDDM and for controls ($r = 0.67$, $p < 0.001$ and $r = 0.43$, $p < 0.01$, respectively). This cannot be explained by our data. It is known, however, that conditions such as chronic renal disease may influence RBP saturation. Indicators of renal function were not measured; it is possible that some subjects with NIDDM has diminished renal function causing the metabolism of RBP to be altered. It is interesting that RBP, but not retinol, was related to the metabolic control of diabetes. Havivi et al. (1991), in contrast, did find a significant positive correlation between retinol and glucose.

Diabetes has been associated with hyperzincuria in subjects with NIDDM (Kinlaw et al., 1983; Schlienger et al., 1988; Melinkeri et al., 1990; Walter et al., 1991; Car et al., 1992; Honnorat et al., 1992) and zinc deficiency is known to reduce the synthesis and release of RBP into circulation (Smith et al., 1973; Smith et al., 1974; Brown et al., 1976; Michaelsson et al., 1976; Shingwekar et al., 1979). Moreover, NIDDM is associated with increased excretion of RBP due to proximal tubular dysfunction (Bernard et al., 1982; Holm et al., 1993; Koh et al., 1993; Holm et al., 1994). Therefore, one might expect to find lower levels of RBP in subjects with NIDDM. Hyperinsulinemia may be protective in terms of ensuring normal plasma levels of RBP and transport of retinol to the target tissues.

CONCLUSION

In summary, metabolic availability of retinol is normal in this group of First Nation people with NIDDM. Impaired metabolism of vitamin A may not occur in NIDDM characterized by insulin resistance, without insulin deficiency. Some populations with NIDDM may have more reduced insulin secretion. Studies are therefore needed to examine retinol and protein status, in terms of insulin availability, within other populations. Future studies are needed to elucidate the role of insulin on hepatic synthesis and release of RBP .

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6. GENERAL DISCUSSION AND CONCLUSIONS

Discussion

Micronutrients are key components of macronutrient metabolism and they act as cofactors and constituents of enzymes. In spite of possible influences of the diabetic state on metabolism, absorption, and excretion, the status of various vitamins and minerals in subjects with diabetes mellitus (DM) and their possible contribution to the expression of the disease has been poorly studied. This is especially true of vitamin A, despite a possible link between vitamin A deficiency and DM in that both cause blindness. Impaired metabolic availability of vitamin A has been found in insulin dependent diabetes mellitus (IDDM) (Basu, 1989); however, it is not known if non-insulin dependent diabetes mellitus (NIDDM) also affects vitamin A status. This study was undertaken to investigate vitamin A status, along with the overall nutritional status, of Plains Cree adults with and without NIDDM, from four First Nations located in central Alberta.

All subjects with NIDDM, except one, had received diabetes instruction and the average duration of diabetes was 9 to 10 years. Dietary instruction appeared to be successful in initiating dietary changes. In fact, 85 % of these subjects reported making a dietary change after diagnosis and the majority reported a reduction in intake of sugar and fat. Dietary changes were reflected in healthier reported dietary intakes for those with NIDDM, based on three non-consecutive 24-hr food recalls and a vitamin A food frequency questionnaire. Subjects with NIDDM had a lower risk of inadequate intakes of vitamin A, D, calcium, folate, and zinc compared to controls. Total fat per megajoule (MJ) was significantly lower and dietary fiber and starch per MJ were significantly higher for women with NIDDM compared to control women. Compared to control men, men with NIDDM also had significantly higher dietary fiber intakes per MJ and lower fat intakes, although the latter was not statistically significant. Sugar intakes per MJ were

significantly lower for men with NIDDM compared to control men, and both men and women with NIDDM consumed significantly less daily energy from the "sweets" food group compared to controls.

In general, fat intakes were above recommended levels and fiber intakes were low. Results from the three 24-hr food recalls confirmed that the diet of these Plains Cree people is now based on commercial foods, with low consumption of traditional foods such as game meats, birds and berries. Their traditional diet was high in protein, low in fat and relatively low in carbohydrate, and the carbohydrate consumed was mainly complex and unrefined. This is in sharp contrast to their current diet which is high in refined carbohydrate and simple sugars, high in fat, and relatively low in protein. Furthermore, their modern diet is limited in dairy products, fruits and vegetables; more than 50 % of participants were at risk of inadequate intakes of vitamin D, calcium, folate, and vitamin A (based on the 24-hour food recall method).

Although many individuals were at risk of inadequate intakes of vitamin A, based on the probability method (Table 4.6), only one subject had a marginal plasma retinol level and none were deficient in retinol. Marginal and deficient plasma levels for vitamin A are defined as 0.70 μM and 1.05 μM, respectively (Gibson, 1990). Plasma retinol concentrations are homostatically controlled and only start to decrease when liver retinol concentrations fall below 20 μg/ g liver (Olson, 1984). Fewer subjects were at risk of inadequate intakes based on the food frequency method than based on the 24-hour recall method, an observation which agrees with the plasma retinol data. Thus, the food frequency method of estimating vitamin A intake appears to be more valid than the mean of three non-consecutive 24-hr food recalls, which is in agreement with previous literature (Russel-Briefel, 1985).

The percentage of subjects with zinc levels below 10.71 μM was 6.8 % and 4.3 %, respectively, for subjects with and without NIDDM. Plasma zinc is also

homostatically controlled, and levels below 10.71 μM is considered deficient (Gibson, 1990). Protein intakes were within the normal range, and few subjects were at risk of inadequate protein intakes. Mean plasma total protein concentrations were similar among those with and without NIDDM and were slightly higher than the normal range, 60 - 80 g/L (Rakel, 1992); 84.2 and 84.4 g/L, respectively, for those with and without NIDDM. A study of Cree in Quebec also found serum total protein values higher than the non-native Canadian sample (Hoffer et al., 1981). Sufficient protein intakes helped to ensure adequate zinc intakes, since these nutrients often occur in the same foods. Consumption of protein and zinc were highly correlated, $r = 0.9209$, $p < 0.001$.

Daily energy consumption was not significantly different between people with and without NIDDM; this was reflected in similar body measurements between these groups. However, although obesity was prevalent, energy intakes were low compared to reference groups. Body mass index estimates health risk for chronic diseases, including diabetes, based on body weight and height. Based on this measurement, a large proportion of the control sample are at risk of developing diabetes. This measure does not distinguish between increased weight due to muscle or fat. Measures of body composition, triceps skinfold, and fat distribution, waist hip ratio (WHR), confirmed that the extra weight was due to excess fat and not muscle. Waist hip ratio measures central body fat distribution and is a better measurement for assessing risk of developing NIDDM than BMI. Based on this measure, the majority of women had central fat distribution. Fewer men than women had central fat distribution, and are therefore at lower risk of NIDDM. Waist hip ratio was also a better indicator of metabolic control than BMI. Waist hip ratio, but not BMI, correlated with blood glucose levels ($r = 0.3468$, $p < 0.05$).

Although insulin sensitivity was not measured, it is suspected that this population has the type of NIDDM associated with insulin resistance. Although the

plasma insulin levels of subjects with NIDDM were similar to control levels, metabolic control of diabetes was poor, especially in the group treated with insulin. The subjects with NIDDM exhibited other characteristics of the insulin resistance syndrome, namely abdominal obesity, hypertriglyceridemia, and hypertension (Davidson, 1995).

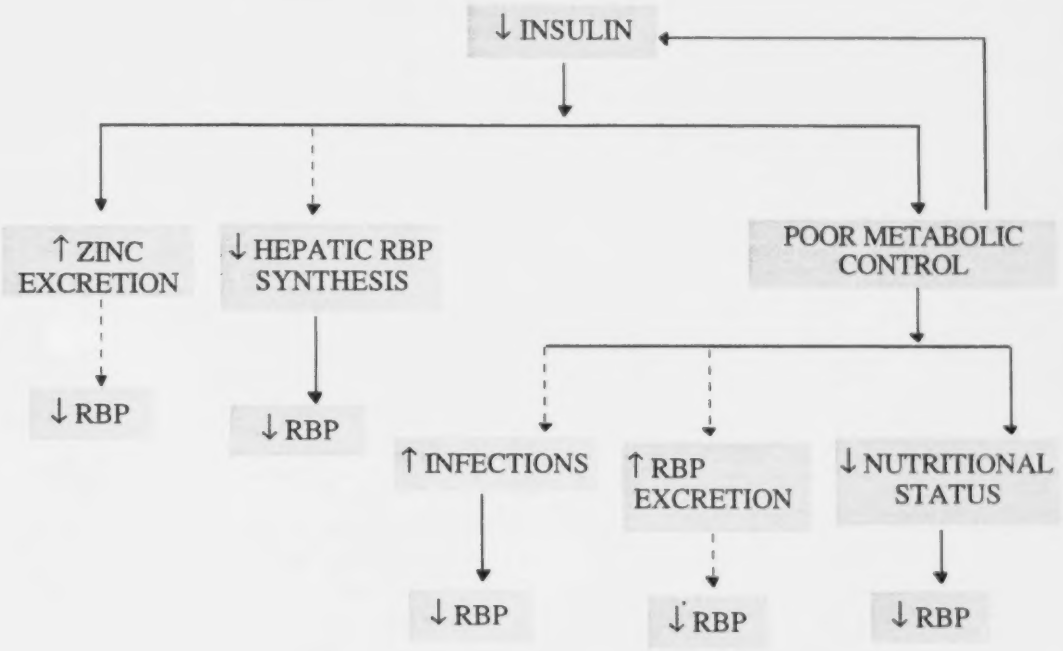
Other indigenous groups worldwide also have a high prevalence of NIDDM associated with insulin resistance, which has been found to be important in the etiology of NIDDM in these groups. (O'Dea, 1991; Bogardus, 1993, Zimmet et al, 1990). It has been hypothesized that, during a traditional lifestyle of feasting and fasting, there was selection for metabolic adaptations that improved survival. Neel (1962) originally proposed this theory and other researchers have expanded on it. Advantageous metabolic adaptations would have been 1) active hepatic gluconeogensis not sensitive to suppression by insulin and a high capacity for hepatic lipogensis sensitive to stimulation by insulin; and 2) an efficient system for fat accumulation including resistance to glucose lowering actions of insulin and normal or near normal sensitivity for insulin action promoting fat deposition (O'Dea, 1991). On a high protein diet, such as that dominated by game animals consumed in traditional times, these adaptations would encourage conversion of amino acids to glucose and fatty acids, and increased hepatic output of triglycerides. Insulin would promote fat deposition in the adipose tissue as blood glucose levels remained high. Physical activity (from hunting and gathering food) would promote insulin sensitivity in the muscles. In modern times, in the presence of a high carbohydrate and high fat diet, these metabolic adaptations have led to high blood insulin levels and a longer glycemic response. Fatty acid and triglyceride production is increased, promoting obesity which further aggravates the insulin resistance. In addition, the lack of physical activity has further reduced insulin sensitivity in the muscles. As high fat, high energy foods have become more readily available and lifestyles have become more sedentary, these metabolic adaptations have "unmasked" a genetic susceptibility to diabetes (O'Dea,

1991).

Thus, the type of diabetes in this population is a result of an inability to compensate for insulin resistance, and not of insulin deficiency *per se*. This is the important difference between this type of NIDDM and IDDM. Insulin dependent diabetes mellitus is characterized by the absence of insulin, due to destruction of the insulin producing cells of the pancreas. Daily insulin injections are required in IDDM to control blood glucose levels. However, in NIDDM, insulin may still be produced but does not function efficiently, for example, there may be circulating insulin antagonists such as hormones or their may be a target tissue defect in insulin action (i.e., insulin receptor kinase defect) (Olefesky and Nolan, 1995). Blood sugar levels can often be controlled through weight loss and dietary modifications, and/ or use of hypoglycemic drugs taken orally. Insulin injections are sometimes necessary for control of NIDDM; however, their use is controversial because they may cause weight gain, causing the insulin resistance to worsen (Koivisto, 1993).

Previous studies found that circulating retinol levels were diminished in patients with IDDM (Wako et al., 1986; Basu et al., 1989; Kempf et al., 1991; Martinoli et al., 1993) and in streptozotocin-induced diabetic rats (Basu et al., 1990; Leitcher et al., 1991; Tuitoek et al., 1996a). In both cases, levels of the vitamin A carrier protein, retinol binding protein (RBP), were also reduced (Basu et al., 1989; Basu et al., 1990; Tuitoek et al., 1996b). Insulin reversed this in the rat model (Leichter et al., 1991; Tuitoek et al., 1996c). Insulin appears to be the key to normalize the metabolic availability of retinol in IDDM. The mechanism has yet to be confirmed; however, several potential mechanisms may be involved (Figure 6.1).

Figure 6.1. Summary of potential mechanisms linking circulating insulin and retinol binding protein in IDDM.



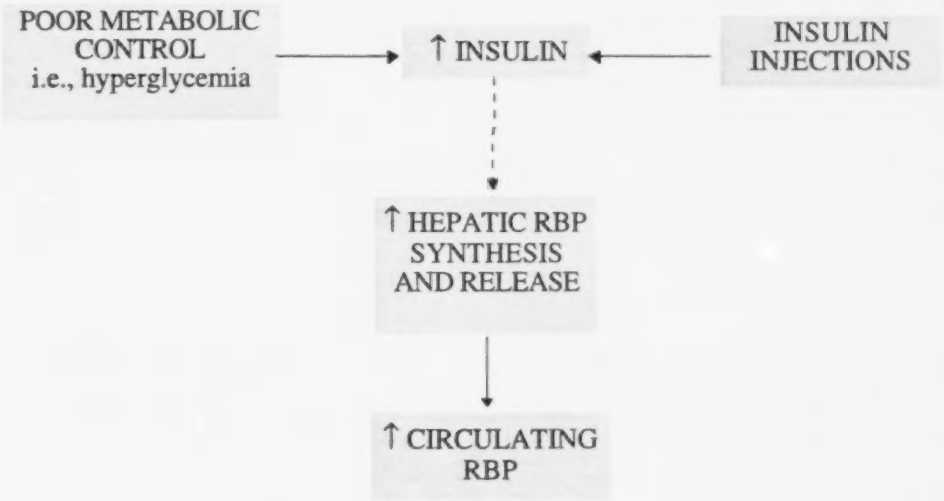
Solid lines represent causal relationships well supported by scientific literature. Dotted lines represent hypothesized causal relationships that are unconfirmed or controversial. Abbreviations: RBP, retinol binding protein; ↑, increasing amounts; ↓, decreasing amounts.

Insulin is an anabolic hormone, capable of promoting synthesis of RBP and mobilization of retinol from the liver (Bowles, 1967). Retinol binding protein may be more sensitive to insulin levels than other proteins produced in the liver because of its short half life. Retinol binding protein's half life is only 12 hours, compared to albumin's half-life of 14-20 days. With less insulin available, the liver may synthesize and release less RBP. Furthermore, insulin is necessary for glucose uptake into insulin sensitive tissues, and in the absence of insulin cells may undergo starvation. RBP is sensitive to nutritional status (Rask et al., 1980) and this starvation may cause a reduction in RBP. Another factor affecting RBP levels may be zinc status. Hyperzincuria occurring in IDDM is well documented and zinc deficiency is know to affect RBP synthesis (Smith, 1973; Hägglöf, 1983). Increased excretion of RBP, due to a defect in the proximal tubules, has also been found (Rowe et al., 1987). Poor metabolic control may exacerbate the situation by reducing the availability of insulin, increasing risk of infection which may also affect nutritional status, and increasing renal damage and loss of RBP.

The reverse situation appears to be occurring in NIDDM, which lends further support for the theory that insulin is the key in normalizing vitamin A metabolism in diabetes. Higher levels of RBP were found in our sample of First Nations adults with NIDDM compared to those without. This agrees with previous studies that found normal or high vitamin A levels in subjects with NIDDM compared to controls (Havivi et al., 1991; Krempf et al., 1991; Straub et al., 1993). Insulin levels among subjects with and without NIDDM were similar and subjects treated with insulin had the highest plasma insulin levels, although this was not significant. Previous research suggests that insulin resistance, causing impaired glucose disposal, does not impair protein metabolism (Staten et al., 1986). Further studies are required to confirm the effect of hyperinsulinemia on hepatic protein metabolism. However, it is proposed that the significantly higher levels of RBP found in subjects with NIDDM were due to higher levels of insulin (Figure 6.2).

Insulin may be acting to increase hepatic RBP synthesis by stimulating protein synthesis and also may be promoting mobilization of RBP from the liver. High insulin levels may also reduce zinc excretion and improve zinc status (Honnorat et al., 1992). Plasma zinc levels were normal; therefore, zinc is probably not affecting RBP levels in NIDDM.

Figure 6.2. Summary of potential mechanisms linking circulating insulin and retinol binding protein in NIDDM.



Solid lines represent causal relationships well supported by scientific literature. Dotted lines represent hypothesized causal relationships that are unconfirmed or controversial. Abbreviations: RBP, retinol binding protein; ↑, increasing amounts.

In contrast to IDDM, where poor metabolic control may cause insulin deficiency, poor metabolic control in NIDDM may actually produce higher circulating insulin, in those with functioning β-cells, resulting in higher circulating RBP. This is supported by the finding that that subjects with the poorest metabolic control had the highest RBP levels (Figure 5.1). Furthermore, metabolic control, indicated by non-fasting glucose, was a significant predictor of circulating RBP only in those with diabetes. This is in agreement with a study by Gougeon et al. (1994) who found greater whole body protein flux in subjects with NIDDM who had poor metabolic control.

Although RBP was higher in subjects with diabetes, retinol was not, even though RBP and retinol were correlated. One molecule of RBP can carry one molecule of retinol, and therefore a molar ratio close to one is generally expected (Smith et al., 1973). The molar ratio of RBP : retinol was higher in subjects with NIDDM compared controls, indicating a reduced saturation of RBP by retinol. Molar ratios of RBP:retinol were elevated in patients with chronic renal disease (Smith and Goodman, 1971). Indicators of renal function such as glomerular filtration rate were not measured; it is possible that some subjects with NIDDM had diminished renal function, causing the metabolism of RBP to be altered. It is interesting that RBP, but not retinol, was related to the metabolic control of diabetes. A previous study found a significant positive correlation between retinol and metabolic control (Havivi et al., 1991).

Conclusions

We had hypothesized that, in view of the poor metabolic control and early onset of NIDDM in Canadian aboriginal communities, a select group of Plains Cree adults with NIDDM in central Alberta would have impaired transport of vitamin A. We, in fact proved the null hypothesis, and found normal vitamin A status in this sample of First Nations adults. Specifically, we wanted to 1) determine if these adults with NIDDM

have reduced vitamin A and RBP status compared to non-diabetic controls and 2) assess the influence of factors that may affect RBP status including zinc status, protein status, plasma insulin levels, and metabolic control of diabetes. The results of our study and other studies in the scientific literature brought us to the hypothesize that insulin status is responsible for altered vitamin A status in DM. Our sample with NIDDM had sufficient insulin to promote hepatic synthesis and mobilization of retinol. Non-insulin dependent diabetes mellitus is a heterogeneous disease and includes both insulin resistance and pancreatic insufficiency. It is necessary to examine circulating levels of RBP and retinol in different populations to understand the metabolic alterations that are occurring in NIDDM. Studying circulating levels of retinol and RBP in subjects with NIDDM, in conjunction with varying vitamin A status, insulin levels, and metabolic control of diabetes, may provide additional insight into the alterations in vitamin A metabolism that occur in NIDDM. Furthermore, the effect of insulin on the synthesis and mobilization of RBP from the liver needs to be elucidated, along with the other factors that may affect circulating RBP.

The potential contribution of altered vitamin A metabolism to the expression of diabetes needs to be investigated. Specifically, studies need to examine the development of diabetic retinopathy in view of vitamin A status. The effect of vitamin A status on resistance to infections should also be studied, especially since NIDDM is associated with a high prevalence and severity of infections (Rayfield et al., 1982; Bryan et al., 1985). The effect of vitamin A supplementation on raising circulating retinol levels needs to be revisited. In IDDM, mobilization of RBP and retinol from the liver may be impaired and supplements should be given with caution since they may contribute to hepatotoxicity. A dose of 25 000 IU per day in a case report of a subject with uncontrolled diabetes led to hepatotoxicity (Kowalski, 1994).

Animal models that mimic the "thrifty genotype" scenario, such as the Israeli sand

rat (*Psammomus obesus*) and the spiny mouse (*Acomys caharinis*), may be studied to provide insight into vitamin A metabolism in highly susceptible human populations in response to westernization (O'Dea, 1992). Another model, the zucker rat, is prone to obesity and has hereditary insulin resistance. Obese zucker rats have fasting normoglycemia, but postprandial insulin and glucose levels are higher than in lean zucker rats, characteristics analogous to early diabetes (Ionescu et al., 1985). Zucker rats can be made hyperglycemic by reducing β-cell reserve with streptozotocin, producing a model that is analogous to later stages of NIDDM. These models may be compared to streptozotocin-induced diabetes in normal rats, which is more analogous to IDDM.

Another objective of the study was to determine the nutritional status of persons with diabetes and non-diabetic controls from three non-consecutive 24-hr food recalls, a vitamin A food frequency questionnaire and anthropometric measures. The dietary and anthropometric data confirmed that controls may be at risk for developing NIDDM. Additionally, subjects with NIDDM may have poor control of their diabetes, as a result of obesity and dietary habits. Many people were at risk of inadequate intakes of calcium, vitamin D, folate, zinc and possibly vitamin A, suggesting a need for increased consumption of dairy products, and vegetables and fruit. These dietary data emphasize the importance of nutrition education to the entire community as part of a more comprehensive diabetes education program. This First Nation community needs to develop and control programs that are culturally appropriate: it is important that the community have ownership of the programs for them to be successful. Furthermore, it is apparent that many control subjects are at risk of developing the disease at a later date. Screening programs to identify new cases of NIDDM should be implemented. For those who have already developed it, support from both the community and health care workers is necessary to help improve their metabolic control and prevent or delay the onset of complications.

Baseline nutrient intake values were obtained for a First Nations community that is relatively prosperous and where traditional foods are no longer consumed. This data can be used for comparisons with other First Nations communities, or for follow-up studies within this community to evaluate the success of nutrition education programs.

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APPENDICES

APPENDIX 1. SUMMARY OF STUDY AND CONSENT FORM

Summary of Purpose and Procedures Given to Participants

Diabetes is becoming a more serious problem in your community. We are asking you to participate in a study to learn about diabetes and nutrition. For this study, we need both people with and without diabetes, and who are 40 years or older.

If you choose to participate in this study we will first take a blood sample. We will later take some body measurements, such as height, weight, and others, and will ask you about what you ate the day before and a few questions about your health. We will need to interview you two more times several days later because what you eat one day may not be the same as on another day. All information will be confidential and will only be used for this study.

If you decide that a study about diabetes and nutrition is important and want to participate, please sign the attached consent form. You, of course, are free to withdraw at any time during the study.

For further information, please contact Dr. Eleanor Wein, University of Alberta, ph: 492-1799 or the Hobbema Indian Health Services.

Consent Form

I acknowledge that the purpose and procedures of the nutrition study have been explained to me. I am willing to participate in the study and I give permission for a health professional to draw a blood sample. I understand that I may withdraw at any time. I know that I may contact the researchers if I have further questions either now or in the future. I understand that although I will not receive any money from the study, important information about nutrition and diabetes may be obtained. I have been assured that personal records relating to this study will be kept confidential. The results of the study, however, may be published in a scientific or medical journal.

_____ _____

Signature of study participant Date

_____

Signature of witness

APPENDIX 2. DIETARY INTERVIEW

Subject number:
Band membership:
Language spoken:
Interviewer's name:
Date (include day of week):
Age:
Gender:
Study category:

1. Diabetes treated by diet and diabetic pills or insulin (circle which treatment)
2. Diabetes treated by diet alone
3. No diabetes

Part A. 24-hour Food Recall

Suggested dialogue:

"I would like you to tell me everything you ate and drank yesterday, and later the amount of each food and beverage. I have food models here which may help you describe the amounts. Let's start with yesterday morning and think through the main activities of the day, especially where and when you ate."

"First, about what time did you get up yesterday?" (record)

"When did you first eat or drink something?" (record)

"What items did you eat or drink?" (list on form) When necessary, ask questions such as: " Did you put anything on the cereal, on the bread, or in the coffee?" etc.; " When did you eat again?"; "What did you eat?"; "Was there anything else?"

"Now let's go back over this list. Would you please tell me how much of each item you had? First you mentioned (name of item). How much of this did you eat?" (etc.)

"Is this how you usually eat?" (If no) "How was it different?"

24-hour Food Recall Recording Form

Subject number:
Date:

Place	Time	Food or Drink and Brand	Amount Consumed	Day	Food Code	Amount Code	Unit

Part B. Vitamin - Mineral Supplements

Do you take any vitamin or mineral pills? yes _____ no _____ I don't know _____
If yes, what kind? (Insert brand name)
Type of supplement 1. Multivitamins, including vitamin A and zinc
2. Multivitamins with iron and calcium
3. Calcium
4. Iron
5. Zinc
6. Vitamin C
7. Other _____

How many of these do you take? _____ per day or _____ per week

Part C. Food Frequency (high vitamin A foods only)

Suggested dialogue:

"Besides asking what you ate yesterday, we want to know how often you eat foods that are high in vitamin A."

"How often do you eat (name of food)?"

Make a check beside the appropriate response on the chart provided; ask questions such as: "How often do you eat (name of food)" and if the answer is not never, ask "Do you eat (name of food) more than once a week or less than once a week?" and give examples of the category responses so that the participant can choose the category that best reflects his/ her intake. Write in any answers that don't fit a category and/ or any specific information given regarding food choices (e.g., circle butter if participant indicates that he/ she eats butter and not margarine)

"How much of this food do you eat at one time?" (Use food models and write in portion sizes.)

After you have completed the food frequency say: "That completes the food information. Now I need to ask a few questions about your health. Different aspects of your health may affect vitamin A and zinc status."

Vitamin A Food Frequency

Subject number: Date:

Food	1-3x day	4-6x day	1-3 x week	1-3x mo.	4-6x year	1-3 x year	never	Serving size
Liver (from any animal or bird)								
Egg (cooked or raw)								
Carrots (cooked or raw; including in mixed vegetables, stew, soup)								
Squash (yellow or orange) or pumpkin (including pie)								
Apricots or peaches (fresh or canned)								
Dark green leafy vegetables (spinach, dandelion leaves, Swiss chard, romaine lettuce)								
Broccoli								
Tomatoes (cooked, canned, fresh or in spaghetti sauce)								
Iceberg lettuce, cabbage (or other lighter green leafy vegetables)								
Snap beans (green beans) or peas								
Powdered coffee creamer								
Cheese								
Sour cream or yogurt								
Butter or margarine								
Milk (including milk in puddings, coffee, white sauce, etc.)								

What type of milk(s) do you drink (circle the answer): whole (homo), 2%, 1%, skim, undiluted evaporated milk.

Part D. Health Information

1. a) Has a doctor ever told you that you have diabetes? Yes _____ No _____
(If no skip to question 2; note: diabetes may have been diagnosed as "borderline diabetes")

b) How long have you had diabetes?

c) Do you take pills or insulin for diabetes? Yes _____ No _____
If pills, what kind? and how often?
If insulin, what type? and when do you take it?

d) Have you changed how you eat since you found out you have diabetes? Yes __No __

e) Do you eat a specified number of calories per day? If yes, how many calories per day?

f) Are there any foods you are not suppose to eat? Yes _____ No _____
If yes, what are they?

g) Do you eat the same number of meals and snacks each day? Yes _____ No _____
Do you eat at the same time each day? Yes _____ No _____
Were you told to eat the same number of meals and snacks each day, and at the same time each day? Yes

h) Where did you learn about your diet? i) hospital; ii) clinic; iii) health center; iv) other

i) Who taught you about how to eat? i) nurse; ii) doctor; iii) dietitian; iv) other, specify

j) Is there anything else we should know about your diet?

2. Are you taking any pills or other medicine (other than for diabetes)? Yes ___No ___
If yes, what is the medicine for? and what is it called?
When did you last take this medicine?

3. Do you: (if yes, answer how much):
i) smoke cigarettes -How many cigarettes do you smoke per day?
ii) use a pipe -How many 1 oz packages of tobacco do you smoke per week?
iii) chew tobacco -How many 1 oz of tobacco do you chew per week?4. How often

4. How often do you drink alcoholic beverages?
What kind(s) do you drink?
When was the last time you drank an alcoholic beverage?
How much did you drink?

Part E. Anthropometric Data

Height:
Weight:
Waist circumference:
Hip circumference:
Mid upper arm circumference:
Triceps skinfold:
- First
- Second
- Third
- Mean

Skinfold completed by:
Date completed: